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Cultivation of *Pleurotus eryngii* on umbrella plant (*Cyperus alternifolius*) substrate

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Abstract Mycelial growth and mushroom yields of three strains of *Pleurotus eryngii* produced on wheat bran-supplemented umbrella plant (*Cyperus alternifolius*) substrate were assessed using surface brightness, bromophenol blue color reactions, ergosterol and glucosamine contents, and water potential as indicators of strain performance. Mycelial growth was 31%–46% greater, depending on strain, on the umbrella plant substrate compared with the mushroom industry standard sugi (*Cryptomeria japonica*) substrate. Mushroom yields on the first flush were 20%–23% higher, depending on strain, on the plastic bottle-contained umbrella plant substrate. However, yields on the second break were lower from the umbrella plant substrate. Because many growers in Japan only harvest one flush, production of *P. eryngii* on umbrella plant substrate may offer commercial producers an alternative basal ingredient to diminishing supplies of sugi sawdust.

Key words *Pleurotus eryngii* · Mushroom production · King oyster mushroom · Umbrella plant

Introduction

Demand for the king oyster mushroom (eringi), *Pleurotus eryngii* (D.C. ex Fr.) Quel, is increasing rapidly because consumers prize its excellent texture and culinary value. This mushroom was originally cultivated in northern Italy and Switzerland, where it is known locally as cardoncello. Cultivation on an industrial scale began in Japan in 1995. Production of king oyster mushroom was estimated at 2000 t in 1996; by 2001, production increased more than threefold to 10070 t.[1] Current demand for the product in Japan indicates that production will continue its rapid growth for the foreseeable future.

The umbrella plant (*Cyperus alternifolius* L.) is a perennial herb of the Cyperaceae family. It is a fast growing monocotyledonous plant with stems 100 to 250 cm in length and up to 5–8 mm wide. The stems are triangular and unbranched, and possess 15 umbrella-shaped leaves at the apex. This plant was recently grown without soil in a floating culture that was established for crop production and water purification in eutrophic natural waters.[2] The umbrella plant is widely planted, but the biomass is not used commercially. Over 85% of the aboveground biomass is stem that consists primarily of high-density vascular bundle tissues. The plant has been tested for pulp making and it shows good potential for this use.[3]

Substrate used for the commercial production of *P. eryngii* consists mainly of sugi (*Cryptomeria japonica* D. Don) sawdust supplemented with wheat bran. Potential shortages of sugi sawdust have highlighted the need to identify alternatives that may be used for sustainable cultivation of this mushroom in the future. Previous work has identified other species of sawdust and bamboo powder as potential sources of substrate for the cultivation of *P. eryngii*.[4,5] The purpose of our work was to evaluate the suitability of substrate prepared from the umbrella plant for production of *P. eryngii*.

Materials and methods

Cultures

Strains of *Pleurotus eryngii* used in this study were from stock cultures maintained on potato dextrose agar medium (PDA). Strains were originally isolated from fruit bodies obtained from commercial sources in 2000. Strains KS-72, KS-18, and KS-54 originated in Japan, Korea, and the USA,

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respectively. These strains were recognized as especially vigorous and were determined different based on the zone line of dual culture. A 5-mm diameter plug of PDA was used as inoculum for the experiments in Petri dishes. Sawdust spawn [*Fagus crenata* Blume sawdust and wheat bran (5:1, v/v)] was used as inoculum for production trials carried out with plastic bottles.

Culture conditions

The umbrella plant raw material was obtained from Nishinihon Green Center, Fukuoka, Japan, in March 1999. The material was ground in a Wiley mill, and screened with sieves to obtain particle sizes that ranged from 20 to 48-mesh size. Sawdust substrate of *Cryptomeria japonica* was used as a control. The sawdust was also prepared to the same mesh sizes.

Two types of sawdust-based media were prepared Petri dishes for the mycelial growth test and plastic bottles for the mushroom production test. The mixture (wood sawdust/wheat bran 5:1, v/v; moisture content: 65%, 100 g) was placed and compressed in a flat bottom 90-mm diameter high-form Petri dish that provided a 20-mm depth of medium. Plastic bottles (800 ml) containing 500 g of the same sawdust-based medium used in the Petri dish experiments was used for mushroom production. The medium was sterilized by autoclaving at 120°C for 30 min and then allowed to cool to room temperature. Agar plugs (5 mm) or sawdust spawn (10 g) were inoculated at the center of the Petri dish medium or the substrate contained in plastic bottles, respectively.

The inoculated substrates were incubated in the dark at 23°C for 14 days. On day 15, the colonized substrates were exposed to cool-white fluorescent lights (500 lx) for 24 h/day. On day 30 after inoculation, spawn and the uppermost layer of the medium were removed and the temperature lowered by flooding with water at 13°C to stimulate primordial formation. The bottled substrate was transferred to a production room which was maintained at 17°C. Misting was provided to maintain 90% relative humidity (RH) in the production room. Mushrooms were harvested from bottles on days 15 to 20 (first flush) and from days 35 to 40 (second flush) after placement in the production room.

Measurements

Mycelial growth (colony diameter) was measured in Petri dish cultures on day 7 after inoculation. The extent of culture maturity was estimated via four methods: (1) brightness,[6] (2) bromophenol blue (BPB) color reaction,[7] (3) ergosterol,[8] and (4) glucosamine.[9] Measurements were made according to procedures described previously.[6–9] Measurements of culture surface brightness were made on day 20 and sampling was completed on day 30 after spawn inoculation for other analyses. Water potentials (ψ) of the cultures were estimated by the thermocouple psychrometer (Wescor HR-33T microvoltmeter coupled to C-52 F sample chamber).[10] The ψ was measured just before the first fruiting (day 30) and the second fruiting (day 50). All mushrooms were weighed to determine mushroom yield.

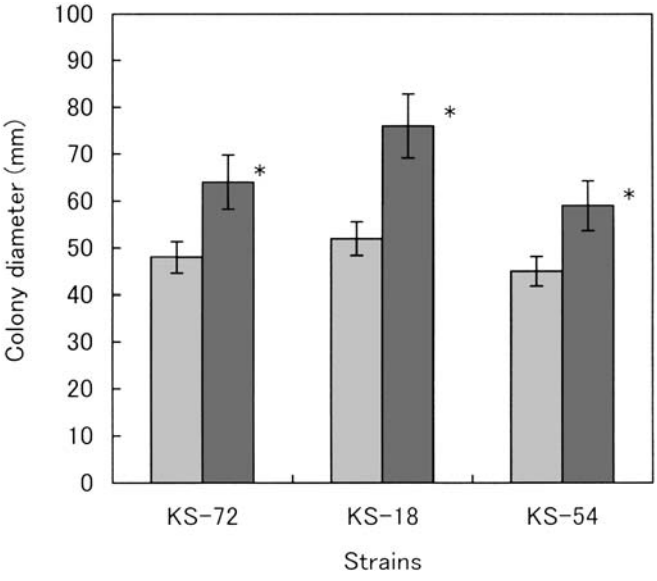

Fig. 1. Mycelial growth (7 days; $n = 10$) of *Pleurotus eryngii* on substrates formulated from *Cryptomeria* and *Cyperus*. *Shaded bars*, *Cryptomeria*; *filled bars*, *Cyperus*. Values are means ± SD. *Asterisks* indicate a statistically significant difference (*t*-test) $^*P < 0.05$

Results and discussion

Mycelial growth

Mycelial growth for all three strains was clearly superior on *Cyperus* substrate when compared with *Cryptomeria* substrate (Fig. 1). Strains KS-18, KS-72, and KS-54 showed mycelial growth 146%, 133%, and 131% higher on the *Cyperus* substrate compared with the control *Cryptomeria* substrate, respectively.

Culture maturity

Mean brightness readings of the substrate surface are shown in Fig. 2. The *Cyperus* substrate showed higher readings and thus greater mycelium growth than the control *Cryptomeria* substrate. Brightness of the substrate surface has a high positive correlation with mycelial density in the early culture phase of growth.[6]

As shown in Fig. 3, b^* values (a measure of the degree of wood decay) were higher (indicating more decay and more vigorous mycelial growth) on the *Cyperus* substrate compared with the *Cryptomeria* substrate. The BPB stain method is a useful technique for estimating culture maturity.[7] This test is based on the response of BPB where it develops a yellow color at pH 3.0 and bluish purple color at pH 4.6. A shift toward the yellow spectrum results in higher b^* values. Higher b^* values are known to correlate with a higher level of substrate decay.

Ergosterol contents of the substrates are shown in Fig. 4. The *Cyperus* substrate contained much more ergosterol than

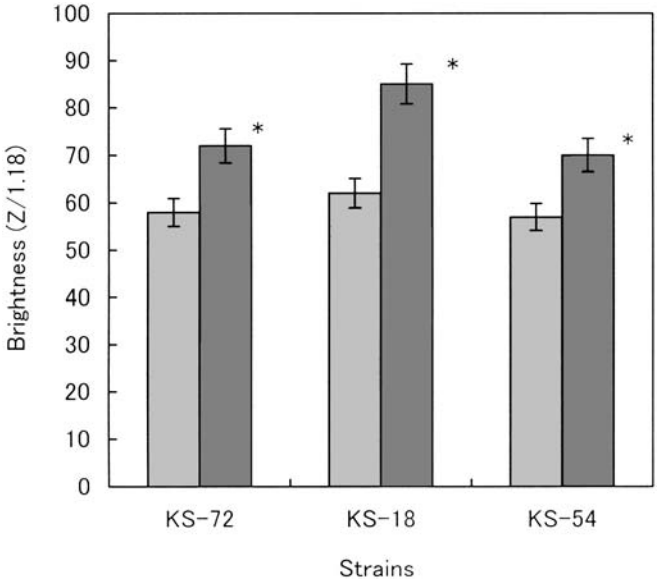

Fig. 2. Surface brightness of the substrate (20 days incubation; $n = 20$) of *P. eryngii*. *Shaded bars*, *Cryptomeria*; *filled bars*, *Cyperus*. Values are means ± SD. *Asterisks* indicate a statistically significant difference (*t*-test) $^*P < 0.05$

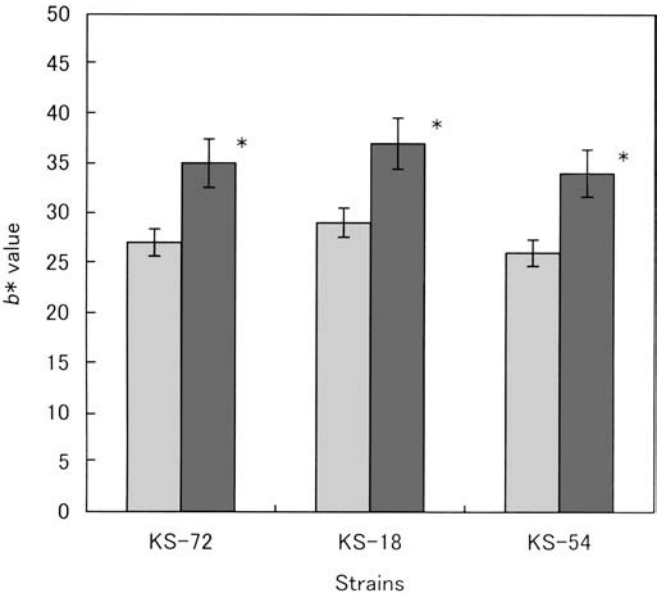

Fig. 3. Values of *b** for bromophenol blue color reaction of *P. eryngii* substrate (30 days incubation; $n = 20$). *Shaded bars*, *Cryptomeria*; *filled bars*, *Cyperus*. Values are means ± SD. *Asterisks* indicate a statistically significant difference (*t*-test) $^*P < 0.05$

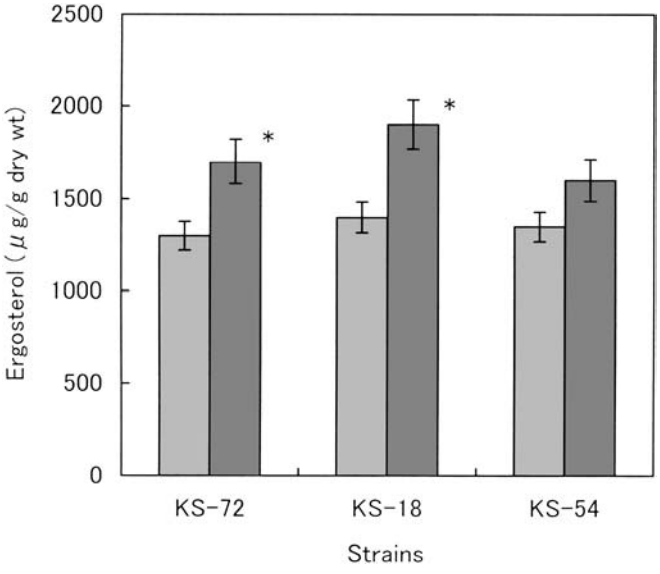

Fig. 4. Ergosterol content of *P. eryngii* substrate (30 days incubation; $n = 20$). *Shaded bars*, *Cryptomeria*; *filled bars*, *Cyperus*. Values are means ± SD. *Asterisks* indicate a statistically significant difference (*t*-test) $^*P < 0.05$

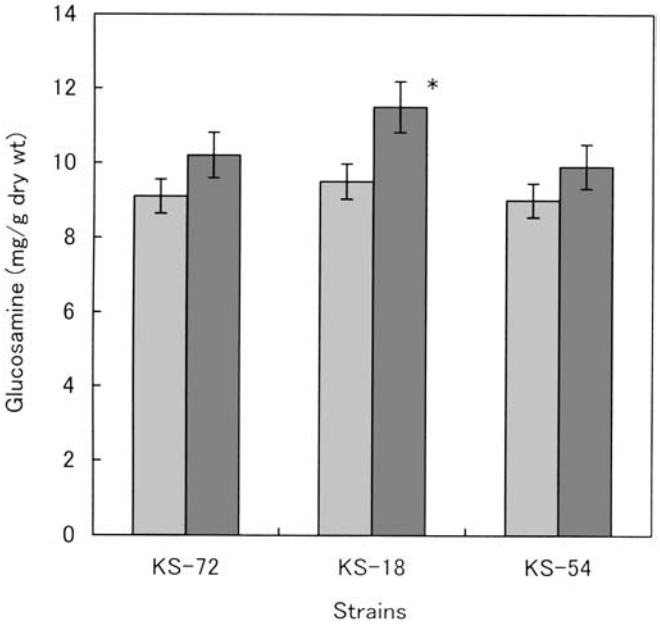

Fig. 5. Glucosamine content of *P. eryngii* substrate (30 days incubation; $n = 20$). *Shaded bars*, *Cryptomeria*; *filled bars*, *Cyperus*. Values are means ± SD. *Asterisks* indicate a statistically significant difference (*t*-test) $^*P < 0.05$

the *Cryptomeria* substrate. As shown in Fig. 5, glucosamine content revealed the same trend, i.e., higher in *Cyperus* than *Cryptomeria* substrate. Ergosterol and glucosamine contents have been used as indicators of substrate maturity in previous work.[4] Levels of egosterol and glucosamine have been reported previously, for vigorous cultures, in the order of 1.3 and 9 mg/g dry substrate, respectively.

Strain KS-18 was the most responsive for the *Cyperus* substrate judging from various culture maturity assessments. The results obtained were brightness 85 (ratio to control: 137%), BPB color reaction *b** value 37 (128%), ergosterol 1900 μg/g (136%), and glucosamine 11.5 mg/g (121%). All measurements showed higher maturity indexes on the *Cyperus* substrate than on the control *Cryptomeria* substrate.

Water condition

Water-holding capacity was quite high (low ψ) in the *Cyperus* substrate at the first fruiting stage (Fig. 6). On the

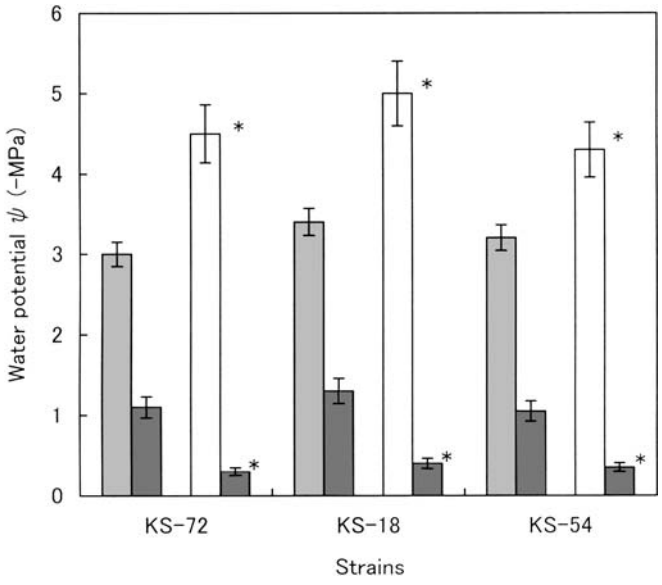

Fig. 6. Water potential of *P. eryngii* substrate ($n = 20$). *Shaded bars*, first flush on *Cryptomeria*; *filled bars*, second flush on *Cryptomeria*; *open bars*, first flush on *Cyperus*; *dense bars*, second flush on *Cyperus*. Values are means ± SD. *Asterisks* indicate a statistically significant difference (*t*-test) $*P < 0.05$

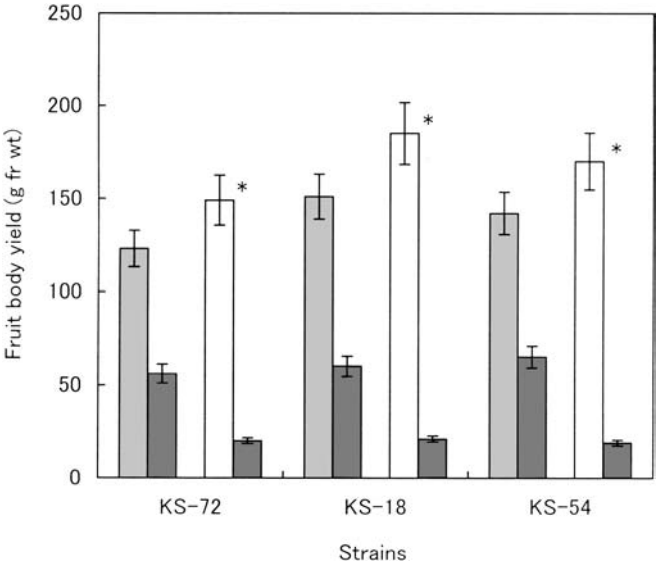

Fig. 7. Fruit body formation of *P. eryngii* on the substrate. *Shaded bars*, first flush on *Cryptomeria*; *filled bars*, second flush on *Cryptomeria*; *open bars*, first flush on *Cyperus*; *dense bars*, second flush on *Cyperus*. The data shown are the averages for 80 bottles. Values are means ± SD. Asterisks indicate a statistically significant difference (*t*-test) $*P < 0.05$

other hand, ψ was very high at the second fruiting stage, which indicates that the water content was too high in the substrate at fruiting.[6] The determination of ψ is the most useful method for estimating water content of the substrate.[10] The values indicate actual water quantity for fruiting in the substrate. Water potential of *Cyperus* substrate may have a larger range capacity for primordial formation than in *Cryptomeria* substrate. Furthermore, the higher availability of water from the *Cyperus* substrate may improve mushroom development and maturation.

Fruit body formation

Mushroom yield was observed for both the first and second breaks. Yields from the first flush were superior on the *Cyperus* substrate compared with those from the *Cryptomeria* substrate; KS-72 (121%), KS-18 (123%), and KS-54 (120%) (Fig. 7). As expected, mushroom yield was positively correlated with mycelial growth and maturity of the substrate as shown earlier.[6] On the other hand, mushroom yield was lower for the second flush on the *Cyperus* substrate when compared with the *Cryptomeria* substrates. This phenomenon might be explained by the larger fruiting on the first flush. For growers, this may not be a concern because many producers only harvest one flush of mushrooms before discarding the substrate.

In conclusion, utilization of *Cyperus alternifolius* for *Pleurotus eryngii* cultivation is promising and has potential commercial application in the mushroom industry. This may, therefore, be a useful substrate that would allow sustainable commercial cultivation of *P. eryngii* in the long term. Research is underway to examine factors such as environmental conditions, substrate handling qualities, and supplements for *C. alternifolius* substrate that will further enhance yields and quality of this increasingly popular mushroom.

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