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ORIGINAL PAPER

Effect of culture parameters on the production of the edible mushroom *Grifola frondosa* (maitake) in tropical weathers

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Abstract Hitherto, little effort has been directed to improve culture conditions for commercial development of maitake (*Grifola frondosa*), an edible and medicinal fungus, due to the short history of cultivation, particularly in tropical weathers. The purpose of this research was analyzing the environmental factors required for successful basidiome development on synthetic substrates in Colombia. We evaluated different cereal grains (corn, barley, sorghum and rice) for spawn production; and industrial by-products (such as coffee spent-ground and oak-sawdust) as substrates for mushroom production. Exploiting these residues for *G. frondosa* solid culturing would primarily provide edible mushroom and simultaneously help in resolving their disposal problem. The use of corn grains as substrate for spawn production results an important factor for reducing crop cycle time. A cold shock to 10°C was requisite for basidiome formation. Coffee spent-ground was a good substrate for mycelial growth, but not for mushroom production. When using oak sawdust plus corn bran as substrate, we obtained consistent yields with combined high biological efficiency (BE) (35.3%), best quality mushrooms, and a crop cycle of 12–14 weeks. The results achieved in this investigation contribute to expand the knowledge on this fungus, and compare favorably with previous works in the northern hemisphere with respect to BE, mushroom quality and crop cycle time.

Keywords *Grifola frondosa* · "Bag-log" cultivation · Mushroom development cycle · Environmental factors

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Introduction

Grifola frondosa (Dicks.:Fr.) S.F. Gray, commonly known as maitake or hen-of-the-woods, a wood decay basidiomycete that naturally inhabits many hardwood species in Asia, North America and Europe, is one of the most popular edible mushrooms. It is a highly nutritious food source and is reported to contain bioactive metabolites which exhibit many medicinal effects including antitumour (Kodama et al. 2003), hypocholesterolemic (Fukushima et al. 2001), antioxidant (Mau et al. 2004) and antidiabetic activity (Horio and Ohtsuru 2001). Maitake commercial production started in Japan in 1981 (Takama et al. 1981; Mayuzumi and Mizuno 1997) with an annually production of 325,000 kg. In 2003 (the latest figures available), 24,900,000 kg were grown in China, the main producing country, and the mushroom ranked 11th among all cultivated species in terms of worldwide annual output (Chang 2005). Maitake production and consumption is also growing fast in the United States (up 38% in 1999–2000; USDA 2000).

Considering the increasing popularity of this mushroom, there are limited reference texts available for cultivating maitake (Stott and Mohammed 2004). Presently, the methods used to grow maitake are mostly adapted from other specialty mushroom cultivation techniques, such as shiitake (*Lentinula edodes*). Bag, bottle and outdoor bed

cultivation are the three basic methods of commercial production of maitake. Commercial production of most maitake is on synthetic substrate contained in polypropylene bags (Shen and Royse 2001). Current knowledge is limited to investigations made especially in the northern hemisphere (Asia and North America) in template and cold areas. As far as we know, there are no studies on the cultivation of *G. frondosa* in tropical weathers.

The purpose of this study was analyzing the environmental factors required for a successful basidiome development on synthetic substrates in tropical weathers. We also evaluated different cereal grains (corn, barley, sorghum and rice) for spawn production; and several by-products of industries in the coffee producing region of Colombia (such as coffee spent-ground and oak-sawdust) as substrates for mushroom production. The major substrate ingredient oak sawdust is a residue from the industry devoted to the production of oak barrels for the liquor manufacturing. Coffee spent-ground, is obtained during the processing of raw coffee powder to prepare "instant coffee". The use of these residues for *G. frondosa* solid culturing would primarily provide edible mushroom and simultaneously help in resolving their disposal problem.

Materials and methods

Organism

Grifola frondosa (PSUMCC 922) was obtained from the Pennsylvania State University Mushroom Culture Collection, USA, and maintained on potato dextrose agar (PDA) at 4°C with periodic transfer. The mycelium was transferred from the stock culture to the center of a Petri dish containing PDA and incubated at 25°C for 21 days. The experiment was conducted under greenhouse conditions, in Manizales Colombia, located at 2,250 m above the sea level, where the average annual temperature is 17°C and the relative humidity (RH) 70%.

Spawn preparation

Five pieces of 1.0 cm^2 of PDA medium colonized by the mycelium were inoculated in a 500 ml glass jar, in which either 3/4, 1/2 or 1/3 of the volume contained grains of either corn, barley, sorghum or rice. Moisture content of the grains was adjusted to 33–40% of the fresh weight. The jars were incubated at 25°C for 25 days, until total substrate colonization. The chemical composition of the grains for spawn preparation and those used for basidiome development were determined by AOAC methods (2000).

Substrate preparation for spawn run, primordial development and basidiome development

Two different formulations were assayed. The first one consisted of (dry weight basis) 75% oak sawdust (25% humidity), 23% corn bran (15% humidity), 1% sucrose (2% humidity) and 1% calcium carbonate. In the second formulation, the 75% oak sawdust was replaced by 50% oak sawdust and 25% coffee spent-ground (70% humidity). The substrates were packed in polypropylene bags and autoclaved at 121°C for 1 h. The humidity was calculated in relation to the components dry weight. Each bag of 32 cm height and 12 cm diameter contained 1 kg of substrate. One square hole of 2.54 cm^2 was made at the top of each bag and covered with a microporous breather strip, to allow for gas exchange. The bags containing the composts were aseptically inoculated with 3% (humidity basis) of spawn. Each treatment consisted of ten or more replicates.

Analysis of the environmental factors required for a successful basidiome development

The effects of different environmental factors required for a successful basidiome development, were also evaluated in each phase. Temperatures (among 4 and 25°C), RH (from 60 up to 80%), light (0–100 luxes) and ventilation requirements, were tested.

Harvesting and determination of biological efficiency (BE) and quality

Mushrooms were harvested from the substrate when the caps were fully mature. The shape and color of the basidiome were used to evaluate the quality of maitake [based on the description of Shen and Royse (2002)]. BE was determined as the ratio of kg of fresh mushrooms harvested per kg of dry substrate and expressed as a percentage (Shen and Royse 2002).

Results and discussion

Effect of different substrates on spawn production and crop cycle time

Grains of: corn, barley, sorghum and rice were assayed for spawn production. The time required for the complete colonization of the different grains varied among 21–29 days. The fastest growth was attained using corn (Fig. 1a), while the slowest was registered with barley (Fig. 1b). In coincidence, when using corn spawn,

colonization of the substrate for basidiome development was faster (26 days for complete colonization), whereas with barley spawn this phase lasted 35 days. Moreover, the orange exudate, which indicates that *G. frondosa* is ready for the induction of primordia, appeared after 70 days with corn, and 80 days with barley. Not only the chemical composition, but also physical factors may have affected the rate of growth. Barley grains are richer in proteins and nitrogen (12.81% and 2.05% dry basis, respectively) and have less fiber content (4.06%), while corn grains have 5.25%, 0.84% and 7.69% of protein, nitrogen and fiber respectively. On the other hand, corn grains are bigger, and a more porous mesh is therefore obtained. Growth of *G. frondosa* seems to be affected by O_2 availability. Hsieh et al. (2006) showed that the mycelial growth of *G. frondosa* was inhibited with less than 40% O_2.

Effect of different substrate formula on spawn run and basidiome development

Previous results indicate that the type of nutritional supplements influence the crop cycle time, yield and basidiome quality (Shen and Royse 2001), but knowledge is currently very limited, due to the short history of maitake commercial cultivation. A common substrate used for

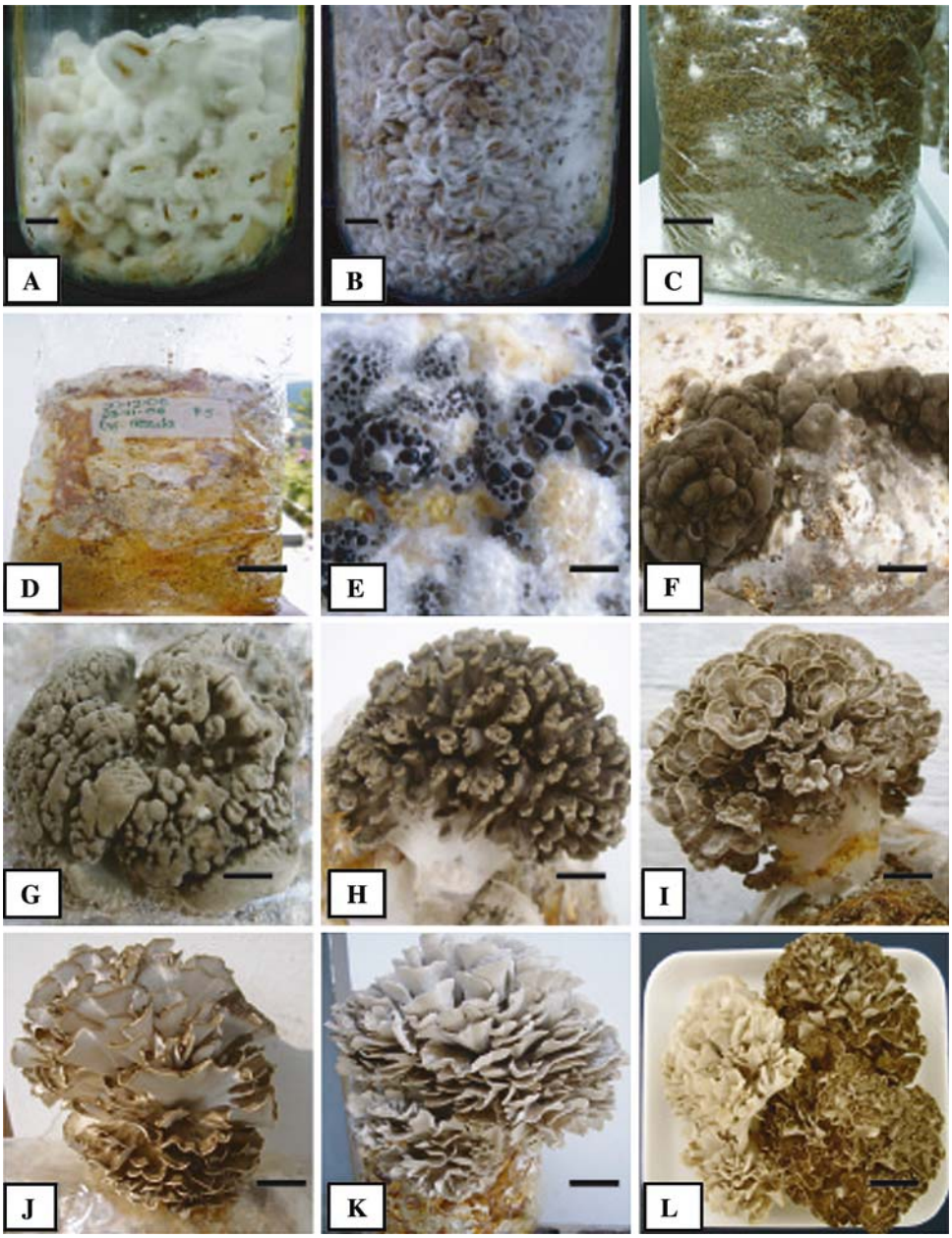

Fig. 1 Maitake spawn on corn (**a**, scale: 1 cm) and barley (**b**, scale: 1 cm); Maitake fruiting body development in oak saw-dust substrate (**c**–**l**): undifferentiated white mycelia (**c**, scale: 2.4 cm), orange brown exudate (**d**, scale: 2.4 cm), dark grey exudate (**e**, scale: 1 cm), uneven topography when primordium stars to develop (**f**, scale: 1 cm), brain stage (**g**, scale: 1 cm), post-brain stage (**h**, scale: 1 cm), cauliflower stage (**i**, scale: 1 cm), cluster flower stage (**j**, scale: 3 cm), mature mushroom (**k**, scale: 3 cm), harvested mushrooms (**l**, left: older mushroom, right: young mushroom, scale: 3 cm)

commercial production is supplemented sawdust. Oak is the most popular choice in the United States and Japan. Brans, derived from cereal grains, such as rice, wheat, oats and corn, are widely used as nutritional supplements (Shen and Royse 2001). The rapid increase of maitake production has emphasized the need to develop more efficient substrate formulas. Two different formulations were assayed in this work. The first one consisted of 75% oak sawdust, 23% corn bran, 1% sucrose and 1% calcium carbonate. In the second formulation, the 75% oak sawdust was replaced by 50% oak sawdust and 25% coffee spent-ground.

Formulations with coffee spent-ground were adequate for mycelial growth but did not develop mushrooms. Coffee spent-ground demonstrated to be a good source for *Flammulina velutipes*, *Pleurotus ostreatus* and *L. edodes* mushroom production (Fan et al. 1999, 2000, 2001). Coffee spent-ground contains caffeine, tannins and polyphenols (Fan et al. 2001). The toxic nature of these compounds may affect basidiome development in *G. frondosa.* On the other hand, considerable differences were detected when quantifying Zn concentration in both substrates [17.3 ppm in oak substrate, and 290.25 ppm when the oak was supplemented with spent-ground coffee (Table 1)]. These differences may also be responsible for the results obtained. White-rot fungi require trace amounts of essential heavy metals such as Cd, Mn or Zn for their growth, but these metals are toxic when present in excess. Toxic heavy metals can inhibit growth, cause morphological and physiological changes and affect the reproduction of Basidiomycetes (Baldrian 2003). The sensitivity of fruiting differs from species to species: *Agrocybe perfecta* failed to produce fruiting bodies during growth on straw with 0.05–1 mM Cd, whereas *P. ostreatus* was much less sensitive (Gabriel et al. 1996). Cd, Hg and Co were also found to be toxic for the sporocarp development of *Volvariella volvacea* (Purkayastha and Mitra 1992). However, taking into account that *G. frondosa* successfully colonized this toxic residue, this substrate formula might be considered for its growth and polysaccharide production.

Table 1 Main components of the substrates assayed for basidiome development

Components	Oak	Oak/coffee spent-ground
Humidity (%)[a]	52.79	52.87
Dry matter (%)	45.21	47.13
Total nitrogen (%)	0.23	0.7
Total Protein (%)	1.44	4.37
Total lipids (%)	0.45	1.18
Fiber brute (%)	52.21	38.69
Total ash (%)	2.4	4.05
P (%)	0.14	0.03
Ca (%)	0.4	0.37
Mg (%)	0.1	0.03
K (%)	0.28	0.14
Na (%)	0.01	0.02
Fe (ppm)	145.47	136.72
Mn (ppm)	29.90	26.25
Zn (ppm)	17.30	290.25
Cu (ppm)	2.10	3.17

[a] Percentages in dry basis

When using only oak sawdust and corn bran we obtained consistent yields with combined high BE (35.3%), and good quality mushrooms (rated 1 on a scale of 1–4 (Shen and Royse 2002), where 1 is the highest quality: basidiomes with black to dark gray color, uniform and no misshapen pilei). The BE is influenced by substrate components, environment throughout the growing cycle, and strain. These results compared favorably with previous results, BE of *G. frondosa* has been found to range from 7.5% to 15.5% (Kirchhoff 1996), 10% to 29% (Stamets 1993), 14% (Chalmers 1994), 16% to 27% (Royse 1997a), 25% to 32.5% (Mayuzumi and Mizuno 1997) with the highest being 35.8% to 39.5% (Shen and Royse 2001). Shen and Royse (2002) when assaying different genotypes of maitake obtained a BE mean for crop I of 34.6%, while total quality mean for crop I was 1.7.

Analysis of the environmental factors required for a successful basidiome development

Compared with other major cultivated mushrooms, very little is known about the parameters affecting the production of *G. frondosa* (Stott and Mohammed 2004). The fungus has a prolonged spawn run. The spawn run is defined as the period from the beginning of inoculation to primordial formation, and may be divided in three stages. Table 2 shows the growth-parameters required for each stage of *G. frondosa* synthetic log cultivation. During the first week (adaptation phase to the new substrate), the cultures were maintained at 25°C and 60% RH, to facilitate a faster growth. Then, they were incubated at 20 ± 1°C for approx. 23–28 days more, until the complete colonization of the substrate. The third step before primordial formation, which includes the development of a dense white mycelial mat (mycelial coat), that progressively acquires an orange brown color, and ends with the production of an orange exudate, required 40–45 days at 18–20°C and 60–65% RH. A cold shock to 10°C for 24 h (induction) was essential to facilitate primordial initiation. Although lower temperatures (4 and 8°C) gave similar results, higher temperatures (up to 15°C) were not effective for primordial induction. While the mycelium could grow in the dark, a low level of light (50–100 luxes) was necessary afterwards for

Table 2 Growth-parameters required for *G. frondosa* synthetic log cultivation in Manizales-Colombia

Environmental parameters	Spawn run (mycelial growth) (in sealed bags)		Primordial formation (in sealed bags)		Fruiting development (in cut-opened bags)
	Sub-phase I (until complete colonization)	Sub-phase II (until exudate formation)	Induction	Primordial formation	
Temperature (°C)	25 (first week), afterwards 20–21	18–20	10	16–18	16–18
RH (%)	60–65	60–65	60–65	70–80	70–80
Light (luxes)	Not required	Not required	Not required	50–100	50–100
Ventilation	Do not requires ventilation	Do not requires ventilation	Do not requires ventilation	One air exchange (10 min) every 12 h	One air exchange (10 min) every 2 h
Time (days)	30–35	40–45	1	7–10	3–5

basidiome development. The reduction from optimum temperature triggers fruit body initiation in many fungi (Stamets 2000). Reports on temperatures required to induce maitake's primordia are conflicting. Chalmers (1994) and Rinsanka (1980) recommended a constant temperature of 20–25 and 22–24°C, respectively, whilst cooler temperatures of 4–18°C (Royse 1996; Mayuzumi and Mizuno 1997; Royse 1997b) or 10–15.6°C (Stamets 1993) have also been indicated. Royse (1997b) also suggested a cold shock at 4°C for 1–4 days was beneficial to initiation (Stott and Mohammed 2004). The period of basidiome development, which was initiated when the primordia begins to grow and differentiate to form small pilei and stipes (7–10 days) required one air exchange (around 10 min) every 12 h, 16–18°C and 70–80% RH. At this stage, holes were cut in the bags exposing the developing primordia. The next phase, until harvesting, lasted for 3–5 days and required an air-renewal of 10 min every 2 h (to hold CO_2 concentrations below 700 ppm) and 70–80% RH. During cultivation of *G. frondosa* recommendations for RH, an important environmental factor, vary between 70% (Mayuzumi and Mizuno 1997), 80% (Kirchhoff 1996) and 95–100% (Stamets 1993). The difference in RH affects substrate moisture content, rate of substrate colonization by hyphae and sporocarp production (Stott and Mohammed 2004). The crop cycle (total time required for spawn run, primordial formation and mature basidiome harvest) obtained in this work was of 81–96 days (until first crop). About 15–20 additional days were required for a second crop. This crop cycle time is comparable to those previously reported by Stamets (2000) and Shen and Royse (2001).

Figure 1c–l shows the fruiting body development in oak sawdust substrate. This process comprise different phases: on day 15 of spawn run an undifferentiated white mycelia can be observed (c); after 75 days a orange brown exudate appears (d); subsequently the mycelial mat (or mycelial coat) covers completely the substrate surface and a dark gray exudate indicates the place where primordia will grow (e). Basidiome development starts with an uneven topography on mycelial surface (f); as the dark grayish black primordia grow, convoluted folds appear on the surface, resembling a brain (this step is described as the brain stage); further growth includes unfolding of the convoluted folds on the surface of the dark primordia into overlapping young pilei (caps) formed in a cluster, followed by elongation of the lateral stems, each with a young pileus (cap) on the upper portion (post brain stage (g)); in the cauliflower stage (i) the developing mushroom cluster has overlapping lighter, almost white petals, with elongated lateral stems each with a young cap on the upper portion, resembling a cauliflower. The next step in basidiome progress is the cluster flower stage (j), where mature fruiting clusters with overlapping petals (caps and lateral stems) extending outward, resemble a cluster flower. At this time, the basidiomes are ready for harvesting (k, l). Maitake mushroom progress from grayish brown when young to light gray, grayish white or light brownish yellow when older (l, left: older mushroom, right: young mushroom).

There is a small amount of research on growth-parameters affecting maitake production, in comparison with other edible fungi such as *Agaricus bisporus* and *L. edodes*, and in particular in the southern hemisphere. Information on cultivation of maitake is often poorly described or has some aspects of commercial-in-confidence, resulting in cultivation methods that are difficult to interpret and implement in a commercial situation. The numerous temperature, humidity and light conditions described lead to further confusion for the new grower (Stott and Mohammed 2004). In comparison with the techniques described by Royse and Guardino (1997), Chen et al. (1999), and Chang and Miles (2004) for maitake cultivation in USA and Japan, which do not emphasize the need of a cold shock for fruiting body development, a decrease of temperature to 10°C was requisite for mushroom formation in the tropical weather of Colombia. In addition, the use of corn grains for spawn production results an important factor for reducing crop cycle time.

In conclusion, the results obtained in this investigation contribute to expand the knowledge on this fungus, and compare favorably with previous works in the northern hemisphere with respect to BE, mushroom quality and crop cycle time. Moreover, although coffee spent-ground was not a good substrate for mushroom production, it was adequate for mycelial growth. Therefore, this residue of the coffee industry might be an interesting alternative for growth and polysaccharide production by *G. frondosa*.

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