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Full Length Research Paper

Characterization and production of shiitake (*Lentinula edodes*) in Mexico using supplemented sawdust

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The cultivation of shiitake in Latin America started during the early 1980's, and several attempts for its commercial cultivation have been carried out during the last decade in Guatemala, Colombia, Mexico, Argentina and Brazil. However, a major constrain has been the lack of basic research, allowing further development. In this work, we studied two genotypes of *Lentinula edodes* (CP-7 and CP-163) selected from 16 strains being used in the region at different levels, in order to assess their mycelial growth rate in Petri dishes, as well as yield (biologic efficiency, production rate) and quality of fruit bodies, using 10 different formulations of supplemented sawdust from a common Mexican oak tree *(Quercus acutifolia* Neé). The best mycelial development was 8.5 mm/day for the genotype CP-163 cultivated on 70% *Quercus* sawdust, 10% corn-cobs, 10% maize stubble, 7% wheat bran and 3% rice meal. The highest yield was recorded in the genotype CP-7, using 60% *Quercus* sawdust, 28.5% corn-cobs, 10% maize stubble, 1.5% gypsum, thiamine (100 mg/kg), and magnesium sulfate (20 g/100 kg); reaching a biologic efficiency of 103%, a production rate of 1.3, and a high proportion (41.8%) of fruit bodies, having good commercial quality (41 to 70 g fresh weight, > 12 cm cap diameter and 96.5% of regular shape). On the basis of this study, this last genotype and formulation was recommended, as well as to establish a breeding program at the molecular level for shiitake production on a large scale in Mexico or other Latin American countries.

Key words: Edible mushrooms, genotypes, substrates, mycelial growth rate, biologic efficiency.

INTRODUCTION

Opportunities for the production of new commodities are being generated worldwide as markets become more globalized. The world-wide production surpasses the 7 million tons of fungi foods cultivated fresh per year, whose approximated economic value surpasses 30 billion dollars. The rate average of annual increase in the production of fungi is superior to 11% (Martínez-Carrera et al., 2007).

Shiitake [*Lentinula edodes* (Berk.) Pegler] is an edible mushroom commonly used as food in Asian countries, and also a traditional Chinese medicine (Lin et al., 2008) which is cultivated on a large scale in many countries (Poppe and Hofte, 1995; Chang and Miles, 2004). It can be produced commercially in Latin America for the world market. Its cultivation in Latin America started during the

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early 1980's, and several attempts for its commercial cultivation have been carried out in Guatemala, Colombia, Mexico, Argentina, and Brazil (Martínez-Carrera, 2002). At present, most research work has been focused on mycelial growth of strains in Petri dish and mushroom yields. Supplemented formulations have been studied containing abundant regional organic materials such as sawdust from *Quercus, Carpinus, Bursera, Alnus, Heliocarpus, Nothofagus, Eucalyptus, Araucaria, Salix,* and *Hevea,* as well as sunflower seed hulls (Mata et al., 1990; Morales and Martínez-Carrera, 1990; Pire et al., 2001). In this work, *Quercus* sawdust was used as substrate since it is an economic material and abundant in some parts of Latin America, which presents a lignocellulosic structure. There is considerable variability in physical and chemical properties of oak sawdust taken from different sources. Sawdust is composed of about 70% carbohydrates (cellulose and hemicellulose) and 27% lignin. Overall, it contains about 50% carbon, 6% hydrogen, 44% oxygen and perhaps 0.1% nitrogen (Starbuck, 1994). The chemical composition of oak sawdust to pH 6.0 (1:5) was: 46.6% total carbon, 0.28% total nitrogen, 166 C/N, 0.07% P_2O_5, 0.22% K_2O, O 0.92% Ca, and 0.11% MgO (Jo et al., 2007).

The biologic efficiency (BE) and the production rate (PR) of shiitake have been evaluated using polypropylene plastic bags containing 0.5 to 1.5 kg (fresh weight) of supplemented oak sawdust (Mata et al., 1990; Sobal et al., 2010). BEs varied from 2 to 112% (Pire et al., 2001; Curvetto et al., 2002). Other investigations have also been carried out to a much lesser extent for cultivating shiitake mushrooms on oak logs (Chang and Miles, 2004; Shimomura and Hasebe, 2004).

Major constrains for further development of shiitake cultivation in Mexico and the rest of Latin America are not only the lack of basic research work, but also the promotion for higher shiitake consumption within the region. At present, shiitake is mostly consumed by gourmet social sectors, as it is a food product which has been recently introduced, produced, and its commercial availability is irregular.

In this work, two genotypes of shiitake were cultivated and assessed using ten different supplemented formulations, based on different concentrations of oak sawdust as substrates in order to generate basic information to establish a breeding programme using molecular tools, and recommend a genotype and a suitable formulation for cultivation on a large scale.

MATERIALS AND METHODS

Genotypes

Two strains of *L. edodes* from the CP culture collection were studied: CP-7 from Hong Kong and CP-163 which has been cultivated commercially in Mexico for many years (Martínez-Carrera et al., 1999). Strains were maintained and stored at 4°C. Standard potato-dextrose-agar medium (PDA; Bioxon), routinely autoclaved at 121°C for 15 min, was used for subculturing strains.

Substrates and formulations

Several substrates readily available in the region, as well as diverse compounds, were used to prepare ten different formulations. Substrates were: different concentrations of oak sawdust (*Quercus acutifolia* Neé), maize stubble, wheat straw, corn-cobs, peanut husk, milled stems from *Tithonia tubaeformis* (Jacq.) Cass, cotton waste, wheat kernels, chopped cardboard, wheat bran, rice flour, and corn flour; while the compounds were: gypsum ($CaSO_4$), calcium carbonate ($CaCO_3$), magnesium sulphate ($MgSO_4$), urea, and thiamine. Different proportions of these materials were mixed thoroughly to prepare every formulation as shown in Table 1. The formulation 1 (37.5% *Quercus* sawdust + 12.5% wheat bran) was used as a standard reference in all experiments, which has also been used in previous research work (Moonmoon et al., 2011; Morales and Martínez-Carrera, 1990; Morales and Martínez-Carrera, 1991). These formulations were taken to a moisture content of 60 to 65%, and then used to grow the strains studied in Petri dishes and polypropylene bags.

Mycelial growth on Petri dish

Fresh substrate (20 g) from each formulation was placed in Petri dishes (90 × 10 mm), and compacted to allow uniform growth. These Petri dishes were sterilized in an autoclave at 121°C for 1 h, and inoculated with the strains studied under aseptic conditions. Inoculated plates were incubated at 25°C in the dark. Experiments were carried out with three replicates, and mycelial growth rates were measured every 3 days. The mycelial growth rates (mm/day) were scored along perpendicular axis across the mycelial colony.

Spawn preparation

Sorghum grain spawn was prepared in jars according to standard methods (Pire et al., 2001). After inoculation, the jars were incubated in the laboratory (26 to 28°C), and shaken at weekly intervals to promote rapid and even growth. Complete colonization varied from 4 to 6 weeks.

Fruiting tests

Selection of better formulations

Strains were cultivated in polypropylene bags with filters to allow gas exchange (25 × 30 cm) containing sterile fresh substrate *(ca.* 1.5 kg) from each formulation. The substrates placed in polypropylene bags were previously sterilized in an autoclave at 121°C for 1.5 h. All bags were inoculated, with strains studied under aseptic conditions, at a rate of *ca.* 100 g/kg fresh substrate weight. Experiments were carried out with four replicates. All bags were taken to an incubation room (24 to 30°C) to allow substrate colonization, and placed completely at random on the shelves.

Bags fully colonized by the mushroom mycelium were transferred to the fruiting room, where ventilation and watering were increased to promote the formation of fruit bodies (minimum temperature: 6 to 17°C; maximum temperature: 21 to 30°C; relative humidity: 70 to 90%). The bags were opened at the beginning of the fruiting and withdrawn when the proportion of mycelial coat brown was 50%. The yield was recorded for one week, and fruit bodies were harvested when mature (fully extended cap). The following data were recorded: period for complete colonization (substrate completely covered by the mycelium), for the formation of the brown mycelial coat (Indicator of maturation of the mycelium) and for the

Table 1. Experimental formulations based on supplemented *Quercus* sawdust, used to assess two genotypes of *Lentinula edodes* (CP-7, CP-163).

Component	Formulation									
	1	2	3	4	5	6	7	8	9	10
Q. acutifolia	87.5	60.0	60.0	40.0	55.0	30.0	65.0	70.0	25.0	25.0
T. tubaeformis	0.0	20.0	0.0	20.0	0.0	30.0	15.0	0.0	0.0	0.0
Wheat bran	12.5	20.0	0.0	17.0	0.0	7.0	10.0	7.0	33.0	18.5
Wheat straw	0.0	0.0	0.0	0.0	0.0	30.0	0.0	0.0	0.0	0.0
Wheat kernels	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0
Corn-cob	0.0	0.0	28.5	0.0	0.0	0.0	0.0	10.0	20.0	0.0
Maize stubble	0.0	0.0	10.0	0.0	0.0	0.0	0.0	10.0	0.0	25.0
Cotton waste	0.0	0.0	0.0	20.0	0.0	0.0	10.0	0.0	0.0	20.0
Peanut husk	0.0	0.0	0.0	0.0	21.5	0.0	0.0	0.0	0.0	0.0
Rice meal	0.0	0.0	0.0	3.0	0.0	3.0	0.0	3.0	0.0	0.0
Thiamine (Vitamin B_1, Roche)	0.0	0.0	100[a]	0.0	100[a]	0.0	100[a]	0.0	0.0	0.0
Magnesium sulphate ($MgSO_4$)	0.0	0.0	20[b]	20[b]	20[b]	0.0	20[b]	0.0	0.0	20[b]
Calciurn carbonate ($CaCO_3$)	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	1.5
Gypsum	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chopped cardboard	0.0	0.0	0.0	0.0	22.0	0.0	0.0	0.0	22.0	0.0
Urea	0.0	0.0	0.0	0.0	0.0	0.0	30[b]	0.0	0.0	0.0

The numbers in the cells are percentages weight/weight. [a]: mg/kg; [b]: g/100 kg.

first harvest. The standard biological efficiency [BE = (Weight of fresh mushrooms / Initial weight of dry substrate) × 100] was calculated without considering the dry weight of the spawn (Tschierpe and Hartmann, 1977).

Assessment of genotypes

The bag size, the amount of substrate and spawn, were changed for this experiment. Strains were cultivated in polypropylene bags with filters (30 × 50 cm) containing sterile fresh substrate (5.5 kg) from formulations 1, 3, 5, 8 and 9 (Table 1). All bags were inoculated under aseptic conditions, at a rate of *ca.* 72 g/kg fresh substrate weight. Experiments were carried out with four replicates. All bags were taken to an incubation room (26 to 28°C) to allow substrate colonization, and placed completely at random on the shelves. Bags fully colonized by the mushroom mycelium were transferred to the fruiting room, where ventilation and watering were increased to promote the formation of fruit bodies (minimum temperature: 6 to 17°C; maximum temperature: 21 to 30°C; relative humidity: 70 to 90%). The yield was recorded for about sixteen weeks, and fruit bodies were harvested when mature. The following data were recorded: period for complete colonization, for the formation of the brown mycelial coat, and for the first harvest. The BE was also calculated without considering the dry weight of the spawn (Royse, 1985). Other variables considered were: production rate [PR = BE / Fruiting period (days)] (Poppe and Hofte, 1995), total number of fruit bodies per formulation, proportions of fruit bodies by weight (< 40 g, 41 to 70 g and > 70 g).

Statistical analysis

Experiments were carried out with four replicates per treatment, using a complete block random design, as well as a factorial design (factors: strains studied and formulation). Analysis of variance was performed and means were separated using Tukey's multiple range test ($p < 0.05$) (Box et al., 1999). Means data are presented in all cases.

RESULTS AND DISCUSSION

Mycelial growth on Petri dish

The growth rates of strains on formulations studied are compared in Table 2. Growth rates varied according to the strain and formulation. The highest growth rate was recorded on formulation 8 for both strains, CP-163 (8.5 mm/day) and CP-7 (7.5 mm/day). These growth rates were not significantly different ($p > 0.05$) than those obtained with the formulation 1 for the CP-163 and CP-7 strains (8.4 and 7.2 mm/day, respectively), and were higher than those obtained by Poppe and Hofte (1995), who reported a mycelial growth of 5.8 mm/day for *L. edodes* grown on sawdust.

Formulations 9 and 3 showed the lowest growth rates for strains CP-163 (3.2 mm/day) and CP-7 (1.8 mm/day). The results show that gypsum and cardboard chopped in the formulations 3 and 9, respectively may be related to the inhibition of mycelial growth. The mycelial growth is attributed to the favorable conditions and advantages of the nutrients for the fungus (Zervakis et al., 2001) as a result of favorable conditions for the activation of the enzyme pack of these fungi (Sanchez, 2009). Although the mycelial growth does not determine a good production of fruiting bodies, this avoids other organisms from colonising the substrate.

Selection of better formulations

The strain CP-7 colonized nine out of the ten formulations,while strain CP-163 showed good

Table 2. Comparison of two genotypes *of Lentinula edodes* cultivated on different selected formulations in the laboratory and experimental chamber.

Experiment	Parameter	Genotype			
		CP-7		CP-163	
		Highest value	Lowest value	Highest value	Lowest value
Mycelial growth on Petri dish	Growth rate (mm/day)	7.5 (8)*	1.8 (9)	8.5 (8)	3.2 (3)
Selection of better formulations	Substrate colonization after 11 days (%)	86.6 (8)	30.0 (10)	90.0 (1, 8)	36.2 (5)
	Substrate colonization after 25 days (%)	100.0 (1, 3, 8)	50 (10)	100 (1, 3, 8)	70 (9)
	Proportion of brown mycelial coat after 62 days (%)	48.3 (8)	26.6 (4)	72.5 (2, 8)	28.3 (4)
	Number of fruit bodies produced	43.2 (1)	1 (4)	20.7 (10)	3.0 (4)
	Total yield (g)	272.1 (8)	30.0 (4)	240.0 (8)	22.8 (4)
	Biological efficiency (%)	61.0 (8)	6.4 (4)	53.8 (8)	4.9 (4)
Assessment of genotypes	Period for harvesting the first flush (days)	160 (8)	130 (1)	135 (9)	122 (1)
	Fruiting period (days)	106 (1)	38 (8)	108 (5)	71 (1)
	Total yield (g)	1,882.3 (3)	315.2 (9)	1,836.2 (8)	388.0 (1)
	Biological efficiency (%)	103.0 (3)	16.8 (9)	102.8 (8)	21.2 (1)
	Production rate (% per day)	2.045 (8)	0.231 (9)	0.993 (3)	0.298 (1)
	Fruit bodies, grade 1: < 40 g (%)	82.1 (8)	58.1 (3)	78.2 (8)	25.0 (1)
	Fruit bodies, grade 2: 41 to 70 g (%)	23.1 (1)	10.0 (9)	30.0 (1)	11.2 (8)
	Fruit bodies, grade 3: > 70 g (%)	23.9 (3)	5.3 (8)	45.0 (1)	7.2 (9)
	Fruit bodies of regular shape (%)	100.0 (9)	86.7 (5)	95.0 (1)	89.1 (9)
	Pileus diameter (cm), colour	10.8 × 11.3, light brown		13.9 × 12.8, dark brown	
	Fruit bodies of irregular shape (%)	13.2 (5)	3.4 (3)	10.8 (9)	5.0 (1)

*The numbers in parenthesis represent the formulations.

Table 3. Average mycelial growth, yield, and biological efficiency of *Lentinula edodes* (strains CP-7 and CP-163) on formulations studied using polypropylene bags (25 × 30 cm) as containers.

Strain	F	Estimated mycelial development (%)*			Fruiting*		
		Colonization after 11 days	Colonization after 25 days	Proportion of brown mycelial coat (62 days)	Number of fruit bodies	Total yield (gr)	BE (%)
CP-7	1	82.5±2.5^{d}	100.0±0.0^{d}	41.6±14.2^{bc}	43.2±1.7^{e}	189.5±59.3^{de}	42.8
	2	65.0±9.5^{c}	92.0±2.5^{bc}	31.6±16.9^{abc}	6.6±2.6^{cd}	144.2±42.6^{d}	31.9
	3	85.0±2.9^{d}	100.0±0.0^{d}	45.0±15.8^{c}	6.6±5.1^{cd}	186.6±52.9^{de}	40.8
	4	32.5±7.5^{ab}	53.7±11.9^{ab}	26.6±4.4 ^{a}	1.0±0.0^{b}	30.0±0.0^{b}	6.4
	5	47.5±7.5^{bc}	93.7±1.4^{c}	35.0±17.3^{abc}	3.3±1.4^{c}	183.1±63.2^{de}	33.8
	6	65.0±11.9^{c}	73.2±16.8^{b}	38.3±4.4^{bc}	4.0±0.0^{c}	53.6±0.0^{c}	11.5
	7	85.0±2.9^{d}	99.0±1.0^{cd}	31.2±4.2^{ab}	3.0±1.1^{c}	137.8±29.7^{d}	30.5
	8	86.6±3.3^{d}	100.0±0.0^{d}	48.3±17.4^{c}	6.0±3.0^{cd}	272.1±46.7^{e}	61.0
	9	37.5±18.9^{ab}	77.5±22.5^{bc}	37.5±7.5^{abc}	8.0±1.0^{d}	160.6±23.9^{d}	34.3
	10a	30.0±0.0^{a}	50.0±0.0^{a}	45.0±0.0^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.0
CP-163	1	90.0±0.0^{e}	100.0±0.0^{c}	67.5±6.9^{d}	9.2±3.0^{bc}	235.0±42.3^{cd}	53.2
	2	60.0±20.0^{ab}	96.6±2.0^{b}	72.5±17.5^{d}	9.5±2.5^{c}	196.0±19.7^{cd}	43.4
	3	80.0±0.0^{cd}	100.0±0.0^{c}	70.0±8.6^{d}	4.5±1.3^{ab}	186.8±23.5^{cd}	40.9
	4	45.2±12.5^{a}	75.0±10.2^{a}	28.3±8.8^{a}	3.0±0.0^{a}	22.8±0.0^{a}	4.9

Table 3. Continued.

5	36.25±15.9^{a}	94.3±2.3^{b}	42.5±2.5^{b}	4.0±3.0^{ab}	158.5±39.5^{bc}	34.4
6	85.0±2.8^{d}	97.2±1.7^{b}	35.0±0.0^{a}	6.5±5.5^{abc}	46.5±30.5^{a}	10.0
7	70.0±10.0^{bc}	97.0±2.3^{b}	48.7±8.0^{bc}	5.5±1.5^{b}	173.6±41.7^{cd}	35.8
8	90.0±0.0^{e}	100.0±0.0^{c}	72.5±8.5^{d}	6.5±0.9^{bc}	240.0±31.2^{d}	53.8
9	60.0±14.1^{abc}	70.0±15.4^{a}	45.0±5.0^{bc}	9.0±7.0^{abc}	95.1±72.4^{ab}	20.34
10	72.5±11.0^{bcd}	95.0±2.8^{b}	62.5±17.1^{cd}	20.7±3.9^{d}	204.6±59.9^{cd}	44.12

F = Formulation; BE = bological efficiency; a = bags contaminated due to poor substrate colonization.*Different letters within each strain and column denote significant differences (P < 0.05); Tukey's student range test. Data are expressed as mean ± SE.

Table 4. Periods for harvesting fruit bodies from strains CP-7 and CP-163 *of Lentinula erodes* using different formulations as substrates.

Strain	F	Period for harvesting the first flush (days)*	Fruiting period (days)*	Total experimental period (days)*
CP-7	1	130±2.9^{a}	106±6.5^{c}	236±7.7^{b}
	3	135±3.2^{a}	79±3.2^{b}	214±6.4^{ab}
	5	131±2.5^{a}	84±3.7^{b}	215±4.9^{ab}
	8	160±4.9^{b}	38±3.3^{a}	198±7.4^{a}
	9	133±3.9^{a}	73±2.9^{b}	206±6.9^{a}
CP-163	1	122±2.5^{a}	71±3.1^{a}	193±6.0^{a}
	3	128±3.1^{a}	83±4.4^{a}	211±7.5^{ab}
	5	126±2.5^{a}	108±6.4^{b}	234±8.9^{b}
	8	128±3.6^{a}	104±4.9^{b}	232±7.9^{b}
	9	135±3.7^{a}	81±3.9^{a}	216±7.4^{ab}

F= Formulation studied. *Different letters within each strain and column denote significant differences (P < 0.05); Tukey's student range test. Data are expressed as mean ± SE.

colonization of all formulations. The period for the shiitake mycelium to colonize substrates, as well as that for the formation of the brown mycelial coat, varied significantly according to every formulation (Table 3). There were three formulations (1, 3, 8) that were completely colonized after 25 days by both strains (CP-7, CP-163). During eight days of mushroom production, which started after 73 days of spawning, the total number of fruit bodies per formulation ranged from 1 to 43 (strain CP-7), while it was from 3 to 20 for strain CP-163. The highest biological efficiencies were recorded in two of these formulations (1, 8) for strains CP-7 (42.8 to 61.0%) and CP-163 (53.2 to 53.8%).

The formulation 8 showed the highest values of mycelial growth rate, substrate colonization, proportion of brown mycelial coat (mycelial maturation), total yield, and BE, in both strains. This result indicates an acceptable proportion of *Quercus* sawdust, wheat bran, corn-cob, and maize stubble. Other components that contribute to improving the development and production of Shitake are peanut husk, thiamine, magnesium sulphate, calciurn carbonate, gypsum and chopped cardboard; included in some of the formulations that showed high values of the indicators evaluated.

On the basis of the joint analysis of mycelial growth rate, maturation of the mycelium, BE, quality of the fungi, and the substrate availability, formulations 1, 3, 5, 8, and 9 were selected for further studies.

Assessment of genotypes

The strains CP-7 and CP-163 colonized all formulations studied. Substrates were firstly colonized 34 days after spawning by the strain CP-7 in formulations 1 and 3. The formation of the brown mycelial coat took around 50 days after spawning for strains CP-7 (formulations 1, 3) and CP-163 (formulations 3, 5, 8). Fruit bodies from strain CP-7 were harvested 130 to 160 days after spawning, while those from strain CP-163 were harvested after 122 to 135 days (Table 4). These data present a number in day superior to the report by Bernabe-Gonzalez et al. (2006) where they reported the initial formation of fruit bodies to be 59 and 61 days, a reason why the studied stocks were slower. The strain CP-7 produced 10 to 56 fruit bodies, mostly of regular shape (86.7 to 100%) during a fruiting period of 38 to 106 days, depending on the formulation studied, while the strain CP-163 yielded 5 to 64.5 fruit bodies during 71 to 108 days, mostly of regular shape (89.1 to 95.0%). The highest yield for strain CP-7 was 1882.3 g in the formulation 3, reaching a BE of

Table 5. Biological efficiencies, production rates, and commercial quality of fruit bodies obtained from strains CP-7 and CP-163 of *Lentinula edodes* cultivated on selected formulations.

Strain	F	N	Total yield (g)	Biological efficiency (%)	Production rate	Quality of fruit body				
						Grading (%)			Shape (%)	
						Grade1 (< 40 g)	Grade 2 (41 to 70 g)	Grade 3 (> 70 g)	Regular	Irregular
CP-7	1	36	1401.9	76.6	0.722	63.9	23.1	12.9	88.9	11.1
	3	39	1882.3	103.0	1.304	58.1	17.9	23.9	96.6	3.4
	5	37.6	1277.9	69.4	0.827	70.8	19.5	9.7	86.7	13.3
	8	56	1388.5	77.7	2.045	82.1	12.5	5.3	94.6	5.3
	9	10	315.4	16.8	0.231	70.0	10.0	20.0	100.0	0.0
CP-163	1	5	388.1	21.2	0.298	25.0	30.0	45.0	95.0	5.0
	3	37.7	1507.1	82.4	0.993	71.5	11.9	16.5	91.4	8.6
	5	32.7	1268.3	68.9	0.638	68.2	15.5	16.3	92.0	8.5
	8	64.5	1836.2	102.8	0.988	78.3	11.2	10.46	92.2	7.7
	9	20.7	668.5	35.8	0.441	77.1	15.7	7.22	89.1	10.8

F = Formulation studied. N = Number of fruit bodies harvested during the fruiting period.

103% and a PR of 1.304 (Table 5). A low proportion of mushrooms harvested were of high commercial quality (23.9%); > 70 g. The strain CP-163 showed the highest yield in formulation 8 (1836.2 g), reaching a BE of 102.8% and a PR of 0.988. A lower proportion of mushrooms harvested were of high commercial quality (10.4%).

On the basis of parameters studied, we can establish that the genotypes CP-7 and CP-163 are closely related, as mushroom yield, BE and mushroom quality, were similar (Table 2). However, differences were observed in the genotype behaviour on every formulation. The highest mycelial growth rates were reached on formulation 8 for both genotypes, but the lowest rates were recorded on formulation 9 (CP-7) and 3 (CP-163). There were also genotype differences for the highest values in the following parameters: the proportion of brown mycelial coat, average number of fruit bodies produced during the period for harvesting the first flush, and the PR (Table 2). In general, fruit bodies from the genotype CP-163 were larger and darker than those from the genotype CP-7. In terms of commercial production, It is recommended to cultivate the genotype CP-7 on formulation 3, combining data from three main parameters: BE (103.0%), PR (1.304), and mushroom quality (41.8% of fruit bodies from grade 2 to 3 and; 96.5% of regular fruit bodies). For the genotype CP-163, formulation 8 was recommended: BE (102.8%), PR (0.988), and mushroom quality (21.7% of fruit bodies from grade 2 to 3 and; 92.2% of regular fruit bodies). These BEs and PRs were higher than those obtained with formulation 1, and those previously reported by Mata et al. (1990), Morales and Martinez-Carrera (1990, 1991), Morales et al. (1991), Ashrafuzzaman et al. (2009), Pire et al. (2001) and Moonmoon et al. (2011).

Conclusion

The best formulations of substrate for mycelial growth and fruiting of the shiitake strains studied were: 70% *Quercus* sawdust, 10% corn-cobs, 10% maize stubble, 7% wheat bran and 3% rice meal (formulation 8) and; 60% *Quercus* sawdust, 28.5% corn-cobs, 10% maize stubble, 1.5% gypsum, thiamine (100 mg/kg) and magnesium sulphate (20 g/100 kg) (formulation 3).

According to the results, shiitake mushrooms can be produced competitively in Latin America on a large scale, using low-cost growing rooms and local formulations based on *Quercus* sawdust. Further breeding work, using molecular tools and involving a genetic base wider than the genotypes available in the region, needs to be established for a sustainable shiitake production.

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