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CULTURE CONDITIONS FOR INCREASING YIELDS OF *LENTINULA EDODES*

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ABSTRACT

The low yields and long cultivation periods associated with traditional shiitake production on logs have been improved by using sawdust substrates and controlled indoor cultivation. Important parameters to increase yields were identified in this study: strain selection and incubation conditions, which avoid browning, cold shock or water immersion at the end of the incubation period. Seven *Lentinula edodes* strains tested (from the USA, Canada and Korea) could be clearly classified in two types of strains. The first group of strains (L5, L9 and L15) are rather high yielding, with biological efficiencies ranging from 143% to 261%. They require high levels of air humidity and produce three flushes of abundant light brown mushrooms in less than 45 days cropping after 70 days incubation at 24°C. A second group of strains (L18, L19, L20 and L21) are lower yielding (B.E. from 24 to 83%) but can fruit at lower air humidity and produce larger and darker mushrooms.

INTRODUCTION

Shiitake production worldwide has increased more than seven-fold from 1983 to 1997 and it is currently the second most cultivated mushroom in the world (Chang 1996, Royse 1997). Most of the increase during this 14 year period occurred in China, where shiitake is widely consumed and exported. Traditionally, shiitake is produced on logs with relatively low yields varying with climatic events, and the whole cycle is very long (three to five years). In the last decades, indoor shiitake production has been introduced in USA and in Europe substituting wood logs by so-called “synthetic media”, sawdust

being the basic ingredient for substrate formulation (Pryzbylowicz and Donoghue 1998). Very strictly controlled conditions of cultivation are required for both mycelium propagation and fruiting, and therefore very costly investments are required.

The indoor method holds two important advantages over natural logs: cultivation time and efficiency. While the natural log cultivation cycle usually lasts six years, only four to six months are normally required from time of inoculation to cleanout for synthetic medium cultivation. Low biological efficiencies (less than 33%) from natural log cultivation are improved to 45 to 65 % using synthetic substrate cultivation (BE = g of fresh mushrooms/100 g dry substrate) (Delpech and Olivier 1991, Rinker 1991, Levanon *et al.* 1993) Recently even higher yields (75 to 125%), have been reported by Royse (1997). To achieve such high yields, bags are removed to expose the substrate blocks after 20 to 25 days spawn-run in an environment conducive to browning of the exterior substrate blocks. As the browning process nears completion after 4 weeks, primordia begin to form about 2 mm under the surface of the substrate, indicating the onset of mushroom production. According to Royse (1997) the substrate has to be soaked in water for 3 to 12 hours to stimulate the maturation of primordia, as water rapidly displaces carbon dioxide contained in air spaces and provides enough moisture for one flush of mushrooms.

Browning and soaking, however, present various drawbacks. Large quantities of water are used; the processes are labor and cost intensive, and the substrate is easily contaminated during these operations. Furthermore, there are contradictory opinions about its usefulness. Extending incubation time to 13 and 15 weeks has been found to preclude the need for browning and soaking although the risk of *Trichoderma* contamination can be increased (Pryzbylowicz and Donoghue 1998). Such contradictory reports may be explained by a genotype effect, since responses to environmental conditions are highly variable depending on strain (Delpech and Olivier 1991, Rinker 1991, Levanon *et al.* 1993). Therefore, in this study various commercial *Lentinula edodes* strains were evaluated for their ability to produce mushrooms without browning and soaking.

MATERIALS Y METHODS

Biological material

Seven strains of *L. edodes* were obtained from various sources: strain L5 from Dr. Ian Reid (National Research Council of Canada), strains L9 and L15 from Dr. Tai-Soo Lee (Forest Research Institute of Korea), strains L18, L19 and L20 from Dr. Rosa L.

Andrade (Instituto Tecnológico Querétaro, México) and strain L21 from Amycel, Inc. (strain 4005). All strains are stored in the culture collection of the Department of Food Science and Biotechnology, Faculty of Chemistry, UNAM.

Culture media

Malt extract agar (MEA) was prepared dissolving malt extract (15%) and agar (2%) in distilled water. It was sterilized in an autoclave at 121°C at 1.2 kg/cm^2 for 30 minutes and ten ml of the sterile medium were poured into sterile petri dishes. Once the medium solidified, the plates were wrapped in plastic bags and incubated at 24°C for two days to check sterility and then used for storage of strains and propagation of mycelium.

Spawn

Wheat grains were washed and cooked for 50 min and then washed again to cool. Excess water was drained, $CaSO_4$ (1.3%) and $CaCO_3$ (0.3%) were added (w/w), 500g supplemented grain were packed in polypropylene bags and autoclave sterilized (2 h at 121°C at 1.2 kg/cm^2). Bags with this sterile grain were inoculated with mycelium from a seven day agar colony and incubated 21 days at 24°C.

Conditions for substrate incubation and fruiting

Sawdust substrate was provided by a commercial grower (Hongos Leben, Edo. Mexico). It was watered to about 54% water content and 2 kg were packed in polypropylene-ethylene bags (32 cm x 49 cm) with a 4 cm x 4 cm micropore filter 13 cm from the top. The substrate was sterilized in an autoclave for two hours at 121°C at 1.2 kg/cm^2. Once cooled, three bags for each strain were spawned (5%) and incubated at 24°C for 70 days. Bags were then transferred to the fruiting room without any further treatment (thermal shock or soaking). Fruiting was induced by regular watering (three times a day for 20 min), two hours ventilation with humid air after each watering period and 12 h illumination per day.

RESULTS

After 70 days incubation, the substrate was fully invaded by mycelium but it lacked a brown cover, since it had remained wrapped in the closed bags during the whole incubation period. On most bags, however, the first primordia had already appeared by this time. Bags were then transferred to the fruiting room and the part of the bags above

the substrate was cut off. The conditions in the fruiting room, i.e. high humidity, regular ventilation and illumination, promoted formation of additional primordia and their maturation into fruit bodies. Nevertheless, developing primordia had to be monitored closely to prevent their death. In order to obtain fruit bodies, some bags had to be placed in the lower racks, close to the floor, where high humidity conditions prevailed. Other bags preferred upper racks where the humidity was lower. Accumulated BEs of the seven *L. edodes* strains are shown in Table 1. Biological efficiencies ranged as follows: 1st flush: 4-138 %, 2nd flush: 17-176 %, and 3rd flush: 24–261 %.

Table 1. Accumulated biological efficiency of *Lentinula edodes* after three flushes.

Strains	Biological efficiency (BE) (g fresh mushrooms/100 g dry substrate)			
	Flushes			
	1st	2nd	3rd	*
L19	4.6 ± 7	17.1 ± 0	24.0 ± 10	a
L18	29.6 ± 23	54.8 ± 25	63.6 ± 17	b
L20	44.8 ± 51	79.2 ± 31	83.5 ± 34	b
L21	53.6 ± 18	70.5 ± 11	78.2 ± 8	b
L5	83.4 ± 7	130.5 ± 5	143.9 ± 11	c
L15	102.5 ± 6	151.2 ± 4	170.1 ± 10	d
L9	138.9 ± 10	176.8 ± 6	261.3 ± 7	e

* Different letters indicate significant differences in BE values at 3rd flush (Duncan's Multiple Range Test p = 0.05 level).

Table 2. Time (days) for completion of each flush after induction of fruiting.

Strains	Time for completion of each flush (days)		
	Flushes		
	1st	2nd	3rd
L19	14	32 ± 11	47
L18	15 ± 2	39 ± 1	51 ± 9
L20	16 ± 2	37 ± 1	51 ± 6
L21	15 ± 2	39	50 ± 1
L5	16 ± 2	28 ± 3	48 ± 3
L15	15 ± 2	31 ± 2	48 ± 2
L9	9 ± 0	22 ± 0	38 ± 1

(Values without standard deviation indicate that only one replicate produced mushrooms for this flush).

Variance analysis indicated significant differences in the data for the 3rd flush. Strain L9 showed the highest productivity (261 % BE), followed by L15 and L5 with 170% and 143 % BE, respectively. Strains L18, L20 and L21 formed a lower-producing group and L19 was the least productive. As shown in Table 2, Strain L9 is not only the most productive but also the most promising in regards to precocity since it completed the three flushes in just 38 days. Strains L5 and L15 are also good candidates for commercial production. In addition to being high-producing they completed the three flushes in just ten additional days. It should be stressed that substrate bags were neither soaked in cold water nor subjected to cold shock to accomplish fruiting. Once spawn run was completed, substrate bags were simply transferred to the fruiting room, where humidity was kept high by fine spraying 20 min, three times a day.

Table 3. Production rates* of seven *Lentinula edodes* strains by flush.

Strains	Production rate (PR) Flushes			Classification by Duncan's test**
	1st	2nd	3rd	
L19	0.06 ± 0.08	0.17 ± 0.01	0.22 ± 0.06	a
L18	0.35 ± 0.27	0.56 ± 0.25	0.55 ± 0.17	b
L20	0.52 ± 0.58	0.81 ± 0.41	0.72 ± 0.27	b
L21	0.63 ± 0.21	0.75 ± 0.01	0.68 ± 0.10	b
L5	0.97 ± 0.10	1.33 ± 0.07	1.22 ± 0.12	c
L15	1.21 ± 0.08	1.49 ± 0.06	1.44 ± 0.07	c
L9	1.76 ± 0.13	1.92 ± 0.06	2.43 ± 0.09	d

* Production rate (PR) = BE/days for obtaining each flush including incubation time

** Different letters indicate significant differences in PR values at all flushes (Duncan's Multiple Range Test p = 0.05 level).

According to values shown on Table 3, Strain L9 was the most productive, continuously increasing its productivity at each flush. Strains L5 and L15 showed high BE values and production rates above 1.0. Strain L15 reached almost 1.5 at the second flush but displayed no further increase at the third flush. Production rates for strains L18, L20 and L21, rather low in comparison to the most productive ones, increased up to the second flush. Finally, strain L19, which had the lowest BE, showed the lowest production rate. Like the most productive strain (L9), however, its production increased with each flush. Statistical analysis indicated the same significant differences among strains after each flush as shown on Table 3, a classification identical to that reported on Table 1 for biological efficiency.

Table 4. Major characteristics of fruit bodies of *Lentinula edodes* strains.

Strains	Quantity			Mushroom size				Brown color		
	low	middle	high	small	medium	large	very lg	very light	light	very dark
L19	■					■	■			■
L18	■					■	■			■
L20	■					■	■			■
L21	■					■	■			■
L5		■			■	■			■	
L15		■			■	■			■	
L9			■	■	■			■		

Table 5. Humidity requirements for fruiting of *Lentinula edodes* strains.

Strains	Relative humidity	Primordia		Fruiting			
		aborted	normal	null	slow	normal	aborted bodies
L19	Low		■		■		
	Middle		■		■		
	High	■		■			
L18	Low		■		■		
	Middle		■		■		
	High	■		■			
L20	Low		■		■		
	Middle	■		■			
	High	■		■			
L21	Low		■		■		
	Middle	■		■			
	High	■		■			
L5	Low	■		■			
	Middle		■			■	
	High		■			■	
L15	Low		■				■
	Middle		■			■	
	High		■			■	
L9	Low	ND	ND	ND	ND	ND	ND
	Middle		■		■		
	High		■			■	

N.D. Not determined

Although the characteristics of the fruit bodies produced by each strain remained fairly constant throughout the flushes, a wide variation was observed between strains. Two types of strains could be identified (Table 4). Within the first group, Strain L9 produced a large quantity of small, light brown mushrooms. Strains L5 and L15 produced a reduced number of mushrooms, but they were a bit darker in color and of larger size. Fewer mushrooms were produced by strains L18, L19, L20 and L21, but they were larger and dark brown. Such variations in morphology and mushrooms yield were associated with two markedly different humidity levels, required by the respective groups of strains. (Table 5). Fruiting in the highest-producing strains (L9, L15 and L5) required high relative humidity. At low ambient humidity, their primordia aborted or deformed fruit bodies were produced. The second group of strains (L21, L20 L18 and L19) produced lower yields at low ambient humidity. At high humidity, primordia aborted and no production was obtained. These strains were also slow producers, probably as a result of the low ambient humidity, which they need for fruiting.

DISCUSSION

Maximum BEs of 60 to 80% have been reported for commercial *L. edodes* production on synthetic substrates (Hiromoto 1991, Royse 2000). Such yields were produced in this study even by the lowest producing strains (63 to 83% B.E.). Before this study, the highest yields reported for *L. edodes* even under laboratory conditions were in the range of 107% to 121% (Royse and Sánchez 2000), but as Table 1 indicates, the high producing strains in this study showed much higher yields (143%, 170% and 261%). One report by Hiromoto (1991), describes even higher yields (B.E. = 325% ± 100) but does not provide details of either substrate composition or growing conditions, citing patent protection. The yields obtained in this investigation, then, are much above those obtained previously.

It is generally accepted that yields are influenced by the type of strain, substrate composition and of course, growing conditions. Spawn running time, mycelial maturation by browning and water soaking of the substrate have been considered as critical factors. Incubation periods from 4 to 15 weeks have been tested with contradictory results (Badham 1988, Delpech and Olivier 1991, Rinker 1991). Controversy exists about the necessity of soaking substrates in cold water. Royse (1997) recommended short periods of two to four hours at 12°C but Stamets (1993) indicated that good fruiting is promoted by soaking for larger periods, i.e. 24 to 48 h, and between flushes. After the long incubation period (ten weeks) used in our study, primordia were

already developing on substrate. At this time bags were removed and substrate transferred to the fruiting room, where high ambient humidity was maintained. This precluded mycelium maturation (browning) and soaking the substrate. The discontinuation of these procedures represents a potential saving of substantial amounts of water and energy for the grower. Badham (1988) proposed increasing spawn-running time to 13 weeks and then transferring the substrate to the fruiting room for substrate hydration. Although the browning and soaking were eliminated, only one flush was cropped in his investigation due to the high incidence of contamination, and low biological efficiencies were achieved (42%). In our study, even the low-producing strains produced comparable results after the first flush, and high-producing strains produced yields two or three times greater (83 to 138%).

The results published by Rinker (1991) indicating that daily misting of substrate was not sufficient to optimize production, and that soaking substrate in water (12 h immersion) was essential for production, may be similarly explained. Yields in that study varied from 25 to 83%, and misted sawdust logs did not produce any mushrooms after the second break. However, these results may be related to genotype. In our study, two of the four low-producing strains yielded even lower BE values for the first flush (ca. 5 and 30%), but strains L15 and L9 showed higher values (102 and 139%). This highlights the influence of the genotype, a factor that has not been adequately considered up to now. Variations in the individual responses of strains to environmental factors have been clearly shown here, and Tables 4 and 5 indicate that the extreme reactions of strains to environmental conditions are associated with their rather opposite characteristics in regards to fruit body morphology.

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