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Shiitake Mushroom Growth on the Formulated Culture Media, Production of Spawn, and Basidiocarps in the Laboratory

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ADDITIONAL INDEX WORDS. *Lentinula edodes*, hardwood sawdust, exotic mushroom

SUMMARY. Culture media YMMBSA (yeast extract, malt extract, multigrain oatmeal, brown sugar, agar), YVMBSA (yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar, agar), and YVMSA (yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose, agar) and broths YVMBS (yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar), YVMS (yeast extract, V-8 vegetable juice, sucrose), and MVBS (multigrain oatmeal V-8 vegetable juice brown sugar) were formulated and demonstrated to be excellent media and broths for growing shiitake mushrooms [*Lentinula edodes* (Berk.) Pegler] in the laboratory. When a portion of the unopened basidiocarp or mushroom fruit (cap or stipe) was isolated on PSA (potato sucrose agar) medium and transferred to the formulated culture media, the mycelia significantly ramified to flocculent

(wooly or fluffy) growth texture within 20 days. For the first time, shiitake mushroom basidiocarps have been induced on the formulated plated media within 20 to 35 days. In tissue culture vessels, mycelia grew well on substrates composed of maple, oak, maple + oak, maple + vermiculite, and oak + vermiculite which had been amended with the broths YVMBS, YVMS or MVBS, attaining spawn texture in 25 to 30 days. Shiitake basidiocarps appeared on the tissue vessels, Magenta GA-7, in 2.6 to 4.1 months. Shiitake mushroom strains, LE1, LE2, LE6, LE7, and LE8, attained flocculent mycelia on the formulated culture media YMMBSA, YVMBSA, and YVMSA in 20 days. Growing the same shiitake strains in the bigger tissue culture vessels, P4928, containing hardwood sawdust amended with broth YVMBS or YVMS or MVBS resulted in significantly larger volume of mycelia growth and spawn texture was attained in 35 to 45 days. Shiitake basidiocarp initials or pins were induced on the spawn blocks in 3 to 5 days after the blocks were squeezed off from the sides of the tissue culture vessels. These results are the first that the formulated culture media considerably enhanced the growing of shiitake mushroom mycelia, production of spawn, and basidiocarps in less time (2.6 to 4.1 months after inoculation) in the laboratory. Basidiocarp productions of shiitake mushroom on amended hardwood sawdust may have an excellent economic potential commercially. It takes 1 to 2 years for basidiocarps to appear in shiitake spawn inoculated logs.

The shiitake mushroom (*Lentinula edodes*) was indigenous to Asia and introduced into the United States in logs by Asian immigrants that settled in the Pacific States in the early 70's (Cook, 1989). It is a wood-rotting and beneficial fungus that belongs to the class basidiomycetes, produces edible basidiocarps or fruit (cap and stipe), and considered an exotic mushroom. Production of the fresh shiitake mushroom crop in the United States in 1995-96 was 6.2 million lbs (2.8 million kg) worth about \$19.8 million (USDA, 1996).

Shiitake mushroom are commonly produced in holed inoculated logs, which basidiocarps typically appeared in ≈1 to 2 years later depending on the shiitake strain used (Donoghue, 1994; Sabota,

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Table 1. Mean separation of shiitake mushroom growth on various formulated culture media for 10 d in the laboratory.^z

Medium used ^y	Growth of isolated shiitake mushroom in various formulated culture media (diam, inches)	Quality of vegetative growth
#48 YVMBSA	2.6 a ^x	Flocculent
#50 YVMSA	2.4 a	Flocculent
#34 YMMBSA	2.4 a	Flocculent
PSA (Control)	2.1 b	Not flocculent

^zThe experiment was replicated three times.

^y#48 YVMBSA = yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar, agar. #50 YVMSA = yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose, agar. #34 YMMBSA = yeast extract, malt extract, multigrain oatmeal, brown sugar, agar. PSA = potato sucrose agar. No mycelia growth was obtained from potato dextrose agar (PDA), lima bean sucrose agar (LBSA), or V-8 vegetable juice sucrose agar (VSA) media.

^xMeans with the same letter are not significantly different according to Tukey's studentized range test (HSD) at $P = 0.05$.

1992, 1994). Before that, it takes ≈ 4 to 6 months to produce spawn (Sabota, 1992, 1994). The mycelia of shiitake mushroom have been grown in 8 different culture media (American Type Culture Collection, 1991). However, these media support growth of the shiitake mycelia quite poorly. Research is currently needed to optimize the growth of shiitake mycelia in the formulated culture media. Also there has been no report of whether shiitake mushroom can grow and produce basidiocarps on amended hardwood sawdust in the laboratory.

The objectives of this study were to 1) develop, screen, and select culture media for growing shiitake mycelia; 2) reduce the amount of time for growing shiitake mycelia and obtain flocculent (wooly or fluffy) growth of the fungus; 3) screen and select substrate for growing shiitake spawn in less time than previously reported; and 4) determine if basidiocarps can be induced on amended hardwood sawdust in the laboratory.

Materials and methods

Experiments on growth of shiitake mushroom mycelia on the formulated culture media and production of spawn of the various strains of shiitake mushroom on the amended hardwood sawdust were arranged in randomized complete block design and replicated three times. Buttons of shiitake mushroom basidiocarp were obtained from a shiitake demonstration plot, courtesy of C. Sabota of the Alabama Cooperative Extension System, Alabama A&M University, to initially isolate and grow shiitake mushroom in culture media. Other *Lentinula edodes* (LE) strains;

LE1 (isolate #48860), a summer fruiting commercial mushroom; LE2 (isolated #48861), a warm weather mushroom strain; LE6 (isolated #48855), a summer fruiting commercial mushroom; LE7 (isolate #48856), a winter fruiting mushroom; and LE8 (isolated #48857), a shiitake mushroom strain that fruit at a wide temperature range; were purchased from the American Type Culture Collection (ATCC), Parklawn Drive, Rockville, Md.

CULTURE MEDIA. The initial culture media used for growing shiitake mycelia were PSA; lima bean, sucrose agar (LBSA); V-8 vegetable juice, sucrose agar (VSA) (Pacumbaba et al., 1992); and potato dextrose agar (PDA) (American Type Culture Collection, 1991). Culture media #34 YMMBSA, #48 YVMBSA, and #50 YVMSA; broths #51 YVMBBS, #52 YVMS, and #59 MVBS were prepared in the laboratory. YMMBSA consisted of 0.3 g yeast extract, 0.3 g malt extract, 52 g multigrain oatmeal, 10 g brown sugar, 10 g bacto-agar, and 1.0 L distilled water. YVMBSA consisted of 0.6 g yeast extract, 60 mL V-8 vegetable juice, 52 g multigrain oatmeal, 10 g brown sugar, 10 g bacto-agar, and 1.0 L distilled water. YVMSA consisted of 0.6 g yeast extract, 60 mL V-8 vegetable juice, 52 g multigrain

oatmeal, 10 g sucrose, 10 g bacto-agar, and 1.0 L distilled water. The broths YVMBBS and YVMS were similar to YVMBSA and YVMSA, respectively, minus bacto-agar. MVBS was similar to YVMBSA minus yeast extract and bacto-agar. Multigrain oatmeal (Quaker Oats Co., Chicago) was ground to a floury texture before being used in the media or broth formulation. The V-8 vegetable juice used is made by Campbell Soup Co., Camden, NJ. Brown sugar is a semi-pure sugar condiment. All the above ingredients except yeast extract, malt extract, sucrose, and bacto-agar are easily available in grocery stores. The above media were autoclaved for 20 min at 15 psi ($6.82 \text{ kg}\cdot\text{cm}^{-2}$) before 10 to 15 mL were poured into disposable sterile petri dishes. The size of the petri dish was $100 \times 15 \text{ mm}$.

ISOLATION OF SHIITAKE MUSHROOM MYCELIA. Mycelial growth of shiitake mushroom was initiated by axenically transferring $0.079 \times 0.079 \times 0.079$ -inch ($2 \times 2 \times 2$ -mm) portions of the unexposed cap or stipe onto PDA, PSA, LBSA, and VSA plated media. Axenic means that the portion of the shiitake mushroom cap or stipe has no contamination with other organisms. This was done by removing part of the surface of the cap or stipe with a flamed scalpel and transferring the exposed portion of the tissue to sterile petri plates containing the media. Inoculated petri plates were incubated at room temperature (RT), 71.6 to 75.2 °F (22 to 24 °C), and observed daily for mycelial growth for 15 d. No mycelial growth of shiitake mushroom was observed on PDA, LBSA, and VSA media. Mycelial tips from the PSA medium were axenically transferred to YMMBSA, YVMBSA, and YVMSA media in petri plates, incubated at RT, and also observed for mycelial growth for 10 d, then for flocculent (wooly or fluffy) growth for another 10 d.

GROWING SHIITAKE MUSHROOM STRAINS ON THE FORMULATED CULTURE MEDIA. Shiitake mushroom strains LE1, LE2, LE6, LE7, and LE8 were each

Table 2. Analysis of variance on the growth of shiitake mushroom on the formulated culture media in the laboratory.

Source	df	Mean square	$P > F$
Medium	3	0.91666667	0.0025**
Replication	2	0.14083333	0.1537
Error	6	0.05416667	

**Significant at $P = 0.001$.

Fig. 1. (A) Mycelia growth of shiitake on culture medium yeast extract, malt extract, multigrain oatmeal, brown sugar, agar (#34 YMMBSA). Basidiocarp initials or pins appeared on these plates 30 d after inoculation. (B) Mycelia growth of shiitake on culture medium yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose, agar (#50 YVMSA). Basidiocarp initials or pins also appeared on these plates 20 d after inoculation. (C) Basidiocarps of shiitake mushroom on plated medium yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar, agar (#48 YVMBSA), 35 d after mycelia inoculation of the plate in the laboratory.

0.20 × 0.20-inch (5 × 5 × 5-mm) agar block portion of the plated 20-d-old culture of shiitake mycelia was axenically transferred to the center of each vessel. The inoculated tissue culture vessels were incubated at RT and observed for growth to attain spawn texture each day for 20 to 30 d. After 30 d, the spawn in the tissue culture vessels was observed for the appearance of shiitake basidiocarps. The above experiment was repeated three times in the laboratory.

The sawdust was a combination of oaks (red and white), maple hickory, tulip poplar, white willow, sycamore, cherry, sweetgum, hophornbeam, american hornbeam/ironwood, ash, osage orange, hackberry, birch, etc. from sawdust of hardwood lumbers, obtained from Moss Lumber Industries, Gurley, Alabama used for the production of spawn in this study. The hardwood sawdust was either fresh or aged (2 to 6 months) passed through a sieve (#H 10/64 × 3/4 inch slotted; Seedburo Equipment Co., Chicago, Ill) to obtain uniform sawdust texture, before placing it in P4928 culture containers. The size of the culture container was 3.5 inches bottom diameter × 4.25 inches high × 4.5 inches top diameter with lid (8.89 × 10.80 × 11.43 cm), (Phytacorn vessels, Sigma Chemical Co., St. Louis, Mo.). To each of the P4928 culture vessels filled with hardwood sawdust, 4.06 fl oz (120 mL) of either YVMBS or YVMS or MVBS broth was added and autoclaved for 20 min at 15 psi (6.82 kg·cm⁻²). To the control culture vessels was added 4.06 fl oz (120 mL) distilled water and autoclaved in the same manner. A 0.20 × 0.20 × 0.20 inch (5 × 5 × 5 mm) agar block portion of the 20-d-old mycelia

grown on the formulated culture media: YMMBSA, YVMSA, and YVMSA and on PSA. The inoculated petri dishes were incubated at RT, observed for mycelial growth for 10 d, then for flocculent growth for another 10 d. The above experiment was replicated three times.

SUBSTRATE USED FOR GROWING SHIITAKE SPAWN. The initial substrates used for growing shiitake spawn were oak (o) and maple (m) sawdust, vermiculite (v), and combinations of two substrates (m+o, m+v, o+v) in a 1:1 ratio and all amended with 2.7 fl oz (80

mL) of broth YVMBS, YVMS, or MVBS. The maple and oak sawdust were obtained fresh locally. The amended substrates or combinations were placed in Magenta GA-7 and autoclaved for 20 min at 15 psi (6.82 kg·cm⁻²). The size of the Magenta GA-7 tissue culture container was 2.95 × 2.95 × 3.03 inches (75 × 75 × 77 mm) (Sigma Plant Cell Culture, Sigma Chemical Co., St. Louis, Mo.). The same volume of distilled water was added to the substrate designated as controls and autoclaved as above. The autoclaved vessels were left for at least 24 h at RT, before a 0.20 ×

Table 3. Basidiocarp productions on sawdust of maple, oak, maple and oak, maple and vermiculite, and oak and vermiculite in the laboratory.^z

Substrate used	Broth used ^y	Length of time (months) for basidiocarp initiations	Total no. of basidiocarps
Maple sawdust	#51 YVMBS	3.4	11
Maple sawdust	#51 YVMBS	3.4	2
Maple sawdust	#51 YVMBS	4.1	4
Maple sawdust	#52 YVMS	2.9	2
Maple sawdust	#52 YVMS	3.4	1
Oak sawdust	#51 YVMBS	4.1	2
Oak sawdust	#51 YVMBS	4.1	1
Oak sawdust	#52 YVMS	3.4	3
Maple + oak	#51 YVMBS	3.4	11
Maple + oak	#51 YVMBS	3.4	16
Maple + oak	#51 YVMBS	3.9	3
Maple + oak	#52 YVMS	2.9	3
Maple + oak	#52 YVMS	3.4	5
Maple + oak	#52 YVMS	3.3	5
Maple + oak	#52 YVMS	3.4	1
Oak + vermiculite	#51 YVMBS	3.4	1
Oak + vermiculite	#51 YVMBS	3.4	1
Oak + vermiculite	#52 YVMS	2.9	3
Maple + vermiculite	#51 YVMBS	3.4	3
Maple + vermiculite	#51 YVMBS	3.6	6
Maple + vermiculite	#52 YVMS	3.1	4
Maple + vermiculite	#59 MVBS	2.6	1
Control	No broth added	4.1	0

^zNo statistical analysis on this experiment due to some substrates combination were not replicated.

^y#51 YVMBS = yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar; #52 YVMS = yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose; #59 MVBS = multigrain oatmeal, V-8 vegetable juice, brown sugar.

was placed in each vessel and allowed to develop spawn texture in 35 to 45 d. To induce basidiocarp initials or pins, each spawn block was squeezed off from the sides of the tissue container to detach the block. The experiment was replicated three times. The formula for volume growth of shiitake mushroom mycelia in P4928 tissue culture vessel is $\pi r^2 h$, where: r = radius of the inside top of the vessel, and h = height of the substrate contained inside the vessel.

All the data obtained were analyzed statistically by either the Tukey's studentized test (HSD) or by ANOVA.

Results and discussion

On PSA medium, an average of 2.1 inch (5.3 cm) in diameter growth of the mycelium occurred 15 d after the $0.079 \times 0.079 \times 0.079$ -inch ($2 \times 2 \times 2$ -mm) portions of the shiitake mushroom cap or stipe of unexposed buttons or pins was plated. No data were obtained from the plated cap or stipe on petri dishes containing PDA, LBSA, or VSA media. The formulated culture media YMMBSA, YVMBSA, and YVMSA supported significantly ($P = 0.05$) mycelial

growth in 10 d with flocculent growth in 15 to 20 d (Table 1). Analysis of variance of the mycelia growth of shiitake mushroom on the formulated culture media was highly ($P = 0.001$) significant (Table 2). Shiitake basidiocarp initials or pins appeared on the same formulated media, 20 to 35 d after inoculation (Fig. 1 A–C).

Spawn texture of the fungus on substrates maple, oak, maple + oak, maple + vermiculite, and oak + vermiculite, amended with YVMBS or YVMS, or MVBS broth was observed in Magenta GA-7 tissue culture vessels, 25 to 30 d after inoculation. No spawn texture was observed on the control tissue culture containers. Mycelia on PSA medium were the original source of shiitake isolate transferred onto YMMBSA, YVMBSA, and YVMSA media. Shiitake mushroom basidiocarps appeared in vessels containing the various combinations of sawdust and vermiculite amended with the broth in 2.6 to 4.1 months (Table 3, Fig. 2A and B). No basidiocarps were observed in the control tissue culture vessels. Similar results were reported earlier

(Pacumbaba, 1994a, 1994b, 1994c).

Shiitake mushroom strains LE1, LE2, LE6, LE7, and LE8 grew significantly ($P = 0.05$) faster on the formulated culture media YMMBSA, YVMBSA, and YVMSA in 10 d and exhibited flocculent growth in 20 d (Table 4). The volume of mycelial growth of the shiitake strains grown in the larger tissue culture vessels (P4928) containing hardwood sawdust amended with any of the broth was significantly ($P = 0.05$) better than the control and attained spawn texture in 35 to 45 d (Table 5). Basidiocarp initials or pins were induced within 3 to 5 d after the spawn blocks were squeezed off from the inside of the tissue culture vessels (Fig. 3A and B). This procedure may have disturbed the vegetative growth of the fungus creating a stress condition, which resulted in the initiation of basidiocarp initials or pins and subsequent basidiocarps. These findings are the first that formulated culture media accelerated the growth and fructification of the shiitake mushroom in a much shorter time in the laboratory. Shiitake mushroom basidiocarp produc-

Fig. 2. (A) Shiitake mushroom basidiocarps appeared on maple + vermiculite (1:1) supplemented with broth #59 (MVBS), 2.6 months after inoculation. (B) Additional basidiocarps of shiitake mushroom appeared on similar substrate or various combinations of substrate amended with broth yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar (#51 YVMSB) or yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose (#52 YVMS) in the laboratory 2.9 to 4.1 months after inoculation.

tures were YM agar/Difco 0712 or YM broth/Difco 0711 and PDA (ATCC, 1991). The ATCC needed more than 50 d to grow the fungus on a PDA agar slant (test tube size was 0.59 × 4.92 inches [15 × 125 mm]) before sending the purchased five strains of shiitake mushroom culture. When transferred to the formulated culture media YMMBSA or YVMSA or YVMSA, the fungus exhibited flocculent mycelia growth within 15 to 20 d. Subsequent propagation and maintenance of shiitake culture strains were made on the formulated media.

Flocculent or fluffy mycelial growth of shiitake mushroom was obtained on the plated formulated culture media within 15 to 20 d. Basidiocarp initials or pins and subsequent basidiocarps were observed for the first time in the formulated culture media. Spawn texture of the fungus was observed on substrates of maple, oak, maple + oak, maple + vermiculite, and oak + vermiculite amended with the various broths in Magenta GA-7 vessels in 25 to 30 d. Basidiocarps also appeared in these vessels in 2.6 to 4.1 months. Shiitake strains grown on the formulated culture media also attained flocculent or fluffy growth in 20 d. Shiitake strains grown on hardwood sawdust in larger tissue culture

tions on amended hardwood sawdust (2.6 to 4.1 months after inoculation) may have an excellent economic potential commercially. Basidiocarp produc-

tion of shiitake mushroom in spawn inoculated logs takes from 1 to 2 years.

The media used for growing and maintenance of shiitake mycelial cul-

Table 4. Growth of shiitake mushroom strains on the formulated culture media in petri dishes for 10 d in laboratory.^z

Medium used ^y	Diam of growth (inches)					Quality of vegetative growth
	Shiitake strain					
	LE1	LE2	LE6	LE7	LE8	
#34 YMMBSA	2.4 c ^x	2.6 abc	2.6 abc	2.5 bc	2.7 ab	Flocculent
#48 YVMSA	2.8 a	2.7 ab	2.8 a	2.6 abc	2.7 ab	Flocculent
#50 YVMSA	2.7 ab	2.8 a	2.7 ab	2.7 ab	2.8 a	Flocculent
PSA (control)	1.6 de	1.6 de	1.7 d	1.4 e	1.7 d	Not flocculent

^zThe experiment was replicated three times.

^y#34 YMMBSA = yeast extract, malt extract, multigrain oatmeal, brown sugar, agar. #48 YVMSA = yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar, agar. #50 YVMSA = yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose, agar. PSA = potato sucrose agar. Lima bean, sucrose, agar (LBSA), V-8 vegetable juice, sucrose, agar (VSA), and potato dextrose agar (PDA) media were not used as controls.

^xMeans with the same letter within the column are not significantly different according to Tukey's studentized range test (HSD) at $P = 0.05$.

Table 5. Spawn of the shiitake mushroom strains on amended hardwood sawdust grown for 40 d in laboratory.^z

Broth used ^y	Vol of growth (inches)				
	Shiitake strain				
	LE1	LE2	LE6	LE7	LE8
#51 YVMBs	297.8 a ^x	301.2 a	295.5 a	298.9 a	301.2 a
#52 YVMS	298.9 a	297.8 a	298.9 a	306.1 a	295.7 a
#59 MVBS	302.3 a	297.8 a	298.9 a	301.2 a	298.9 a
Control	2.2 b	2.8 b	2.3 b	2.4 b	2.3 b

^zThe experiment was replicated three times. Hardwood sawdust were obtained from Moss Lumber Industries. Volume growth = $\pi r^2 h$, where r = radius of the inside top of the vessel and h = height of the substrate contained inside the vessel.

^y#51 YVMBs = yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar. #52 YVMS = yeast extract, V-8 vegetable juice, multigrain oatmeal, sucrose. #59 MVBS = multigrain oatmeal, V-8 vegetable juice, brown sugar.

^xMeans with the same letter within the column are not significantly different according to Tukey's studentized range test (HSD) at $P = 0.05$.

Fig. 3. (A) Spawn texture of the fungus was observed on hardwood sawdust amended with broth yeast extract, V-8 vegetable juice, multigrain oatmeal, brown sugar (#51 YVMBs), 35 to 45 d after inoculation of the tissue culture vessels, P4928. (B) Two-month-old spawn blocks with shiitake mushroom basidiocarp in the laboratory.

vessels (P4928), amended with broth attained spawn texture in 35 to 45 d and basidiocarp initials or pins were induced on the spawn blocks 3 to 5 d

later after the spawn blocks were squeezed off from the inside of the tissue culture containers. These findings are the first that the formulated culture media accelerated the growth and fructification of the shiitake mushroom in a much shorter time in the laboratory. Growing shiitake mushroom and inducing basidiocarp productions on amended hardwood sawdust (2.6 to 4.1 months after inoculation) may have an excellent economic potential commercially. Production of basidiocarps in shiitake spawn inoculated logs takes from 1 to 2 years.

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