International Journal of Biotechnology Research Vol. 2(5), pp. 060-064, July 2014
Available online at http://academeresearchjournals.org/journal/ijbr
ISSN 2328-3505 ©2014 Academe Research Journals

Full Length Research Paper

Molecular characterization and standardization of cultivation for wood ear mushroom [*Auricularia polytricha* (Mont.)] Sacc.

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Accepted 15 July, 2014

Surveys were conducted in the hills of Nilgiris, Shervoys and Lower Pulneys during rainy season. A wood ear mushroom was collected from coffee plantations of Horticultural Research Station, Yercaud. The fungus was identified as *Auricularia polytricha* (Mont.) Sacc. based on cultural and morphological characters. Molecular characterization studies on *A. polytricha* with *P. platypus, P. florida* and *P. eous*, using ITS primers exhibited similarity in ITS lengths among the mushrooms. On gel electrophoresis, all the mushrooms amplified a fragment between 600-700 bp. The studies conducted at Mushroom Research and Training Centre, TNAU, Coimbatore revealed that the paddy straw+wheat bran (3:1) ratio recorded minimum days for spawn run (21.3 days), pin head formation (31.3) and first harvest (35.6 days). The same combination also recorded the highest yield of 147.6 g/bed bioefficiency of 59.04%. The total cropping period was also the minimum in the same treatment. In the trials conducted at Vijaya Mushrooms, Coimbatore (North), paddy straw+wheat bran (3:1) ratio again recorded a significantly higher yield of 132.0 g/bed and bio efficiency of 58.20% with minimum cropping period of 47.3 days. The yield performance trials conducted at Maha Mushroom, Kovaipudur, and Coimbatore (South) also revealed the same trend as paddy straw+wheat bran (3:1) again recorded significantly higher yield of 130 g/bed and bioefficiency of 52.00%.

Key words: *Auricularia polytricha*, substrates, spawn production, ITS primers.

INTRODUCTION

Auricularia polytricha is widely distributed throughout the tropical and subtropical regions of the world (Zoberi, 1972). Nowadays, *Auricularia* mushrooms are among the top four most important cultivated mushrooms in the world, grown mainly in China and Southeast Asia, with a world annual production of 4, 20,000 tons (Yan et al., 1999). Their unique jelly texture and horizontally septated basidium make them significantly different in taste and morphological characters from other cultivated mushrooms such as *Agaricus bisporus, Lentinulla edodes,* and *Pleurotus* spp. Besides their taste and nutrition, they also have medical functions, such as anti-tumour, immuno-stimulating, hypolipidaemic and hypo cholesterol emic effects (Zhang et al., 2006). They can grow well on wide ranges of agricultural and industrial organic wastes. Recently, a nature-imitated cultivation method has been developed in China by cultivating *Auricularia* mushrooms in corn fields, and thereafter, using the spent compost as organic fertilizer and soil conditioner (Yan et al., 2004).

Wood ear mushrooms (*Auricularia* spp.) are commonly cultivated in Asia. Plastic bag cultivation is gaining popularity due to the scarcity of suitable logs and the ease with which different species of *Auricularia* can be cultivated on sawdust. The technology can be expected to spread in the near future. There are many *Auricularia*

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Table 1. Reaction mixture.

Parameter	Value (µl)
PCR master mix	5.0
Primer (Forward)	0.5
Primer (Reverse)	0.5
Double distilled water	3.5
Template DNA	1.0
Total	10.0

species of which *A. polytricha, A. fuscosuccinea* and *A. auriculu-judea* are the most commonly grown. *A. polytricha* is the most suitable species to cultivate in tropical regions where temperatures are high (Well et al., 1984). In the wood ear mushroom, minimum work has been done on selection of best substrate for spawn production, growth, yield performance of *A. Polytricha* and the partial genome sequence. The present study is aimed at finding out the suitability of growing the wood ear mushroom, *A. polytricha*, in Tamil Nadu by low cost substrates and to assess its yield potential at different locations.

MATERIALS AND METHODS

Collection and maintenance of wood ear mushroom

Surveys were conducted in the hills of Nilgiris, Shervoys and Lower Pulneys during rainy season. A wood ear mushroom (*Auricularia* sp.) was collected from coffee plantations of Horticultural Research Station, Yercaud. The fungus was isolated and maintained in the PDA medium for future study.

Molecular identification studies

DNA extraction

The fungal cultures were grown on Potato Dextrose broth for ten days and the mycelium was harvested for DNA extraction. 1 g of the mycelium was macerated in liquid nitrogen using pestle and mortar and was mixed with extraction buffer. The extract was incubated at 60°C in water bath for 60 min. CTAB buffer was added and incubated at 60°C for 10 min. Chloroform and isoamyl alcohol in the ratio of 24:1 were added and centrifuged at 10,000 rpm for 10 min at 4°C. The upper aqueous phase was collected and equal volume of isopropanol was added and incubated in room temperature for 1 h and centrifuged at 10,000 rpm for 10 min and the supernatant was discarded. The DNA pellet was washed in 500 µl of 70% ethanol and centrifuged at 12,000 for 10 min and the supernatant was discarded. The pellet was air dried and dissolved in 20 µl of 1× TE buffer. Agarose gel (0.8%) electrophoresis was run to confirm the presence of total DNA (Singh et al., 2006).

Polymerase chain reaction

The DNA extracted from the mycelium was diluted with 20 µl of 1× TE buffer and amplification was carried out in thermal cycler as shown in the reaction mixture (Table 1).

Primer design (Chromus bio tech Pvt.Ltd)

Forward primer -(5'-TCC GTA GGT GAA CCT GCG G-3').
Reverse primer -(5'-TCC TCC GCT TAT TGA TAT GC-3').

PCR cycles

The reactions were performed in a Mater Cycler with PCR conditions consisting of 40 cycles:

1. Initial denaturation at 92°C for 5 min.
2. Denaturation at 92°C for 1 min.
3. Annealing at 35°C for 1 min.
4. Extension at 72°C for 2 min.
5. Final extension at 72°C for 1 min, with lid heating option at 110°C.

PCR was run at 85 volts. The product obtained was stored in deep freezer at -20°C and two percent agarose gel (0.8%) electrophoresis was run to observe the amplification. 1KB DNA ladder was loaded in one well and samples in other wells.

Growth and yield performance studies of *A. polytricha* at Mushroom Research and Training Centre (MR and TC), Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore

The spawn of *A. polytricha* was prepared on sorghum grain+wheat bran (3:1) substrate. After complete spawn run, beds were prepared following the method described by Sharma et al. (2006) with modifications. The beds were prepared using different substrates namely: paddy straw alone and in combination with saw dust, rice bran, wheat bran at the ratio of 3:1 and 1:1 for comparison. The substrates of different combinations were wetted thoroughly with water for overnight (16-18 h). Two fifty gram of the substrate with 60% of moisture was filled in poly propylene bags of size 12"×12" (80 gauge thickness) and autoclaved at 20 lbs pressure for 2 h. After sterilization, the bags were inoculated with 100 g of spawn each and incubated at 25-28°C. After full mycelial growth, the bags were completely opened and temperature of 24°C and relative humidity of more than 85% was maintained. Three replications were maintained.

The observations on days for spawn run (DFSR), days

for pin head formation (DFPF), days for first harvest (DFFH), days to complete two harvests (total cropping period) and total yield were recorded.

Growth and yield performance studies at different locations

The beds were prepared as mentioned and maintained at the following locations for comparative studies:

(1) Vijaya Mushrooms, Coimbatore (North).
(2) Maha Mushrooms, Coimbatore (South).

The observations on days for spawn run (DFSR), days for pin head formation (DFPF), days for first harvest (DFFH), days to complete two harvests (total cropping period) and total yield were recorded.

RESULTS AND DISCUSSION

Molecular characterization studies of *A. polytricha*

It is difficult to isolate DNA from *Auricularia* strains because their mycelia contain high amounts of polysaccharides. After comparing several methods, CTAB method was slightly modified to obtain good quality DNA from liquid cultures of mycelia. To make mushroom cultivation sustainable and highly productive, novel improved strains with improved characteristics are greatly needed. However, mushroom strains are very difficult to discriminate due to lack of clearly distinguishable characters. Molecular characterization of *A. polytricha* was studied using ITS primers by comparing with *P. platypus, P. florida,* and *P. eous*. The polymerase chain reaction primers, ITS-1 and ITS-4 were used to amplify the ITS of ribosomal DNA, which encompasses both ITS-1 and ITS-4 regions. The results of the study indicated that all mushrooms exhibited similarity in ITS lengths. On gel electrophoresis, the amplified region of *A. polytricha* showed fragment of 600-700 bp. The amplified product was eluted and sequenced.

Growth and yield performance of *A. polytricha*

Growth and yield parameter of *A. polytricha* were recorded at Mushroom Research and Training Centre (MR and TC), Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and also at two selected Commercial Mushroom Farms namely: Vijaya Mushrooms (Coimbatore-North) and Maha Mushrooms (Coimbatore-South).

Growth and yield performance of *A. polytricha* at MR and TC, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore

The results of trial conducted at MR and TC, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore are presented here. The results indicated that mixing of wheat bran and rice bran with paddy straw at 3:1 ratio was found to induce faster growth of *A. polytricha* in beds. However, mixing of saw dust at 3:1 ratio with paddy straw recorded slower growth when compared to paddy straw alone. Total cropping period was minimum (50.6 days) in paddy straw + wheat bran at 3:1. This was followed by paddy straw + rice bran at 3:1ratio (55.0 days). Paddy straw + saw dust (1:1) recorded maximum days (69.3) of cropping period when compared to other combinations.

The beds prepared with paddy straw + wheat bran at 3:1 ratio also recorded maximum mean yield of 147.6 g/bed with the bioefficiency of 59.04%, whereas paddy straw + saw dust (1:1) recorded the lowest mean yield of 107.3 g/bed with the bioefficiency of 42.92%. Paddy straw + wheat bran (3:1) also recorded lower days of spawn run (21.3), lower days for pinhead formation (31.3) and lower days for first harvest (35.60). This was followed by paddy straw + rice bran (3:1). Paddy straw + saw dust (1:1) took longer days for spawn run (46.3), longer days for pinhead formation (51.0) and maximum days for first harvest (55.00), when compared to other substrates (Table 2).

Growth and yield performance of *A. polytricha* at selected Commercial Mushroom Farms of Tamil Nadu

Vijaya Mushrooms (Coimbatore-North)

The trials conducted at Vijaya Mushrooms, Coimbatore (North) also revealed the same trend. The beds prepared with paddy straw + wheat bran at 3:1 ratio recorded maximum mean yield of 147.0 g/bed with a bioefficiency of 58.80%, followed by paddy straw + rice bran (3:1) as it recorded the second highest mean yield of 132.00 g/bed with the bioefficiency of 52.8%. Same trend was also observed in days for spawn run, days for pinhead formation and days for first harvest (Table 3).

Maha Mushrooms (Coimbatore-South)

The second trial was conducted at Maha Mushrooms, Kovaipudur, Coimbatore. In this case also, paddy straw + wheat bran at 3:1 ratio recorded the lowest days for spawn run (DFSR), days for pinhead formation (DFPF) and days for first harvest (DFFH) when compared to other treatments. Moreover, it also recorded maximum mean yield of 130.0 g/bed with bioefficiency of 52.00%, followed by paddy straw + rice bran at 3:1 ratio with bioefficiency of 47.44% (Table 4). Thiribhuvanamala et al. (2005) reported that paddy straw, mixed saw dust and wheat bran substrates resulted in early spawn running with uniform mycelial growth of *A. polytricha* with bio efficiency of 46.4%. Sharma and Puttoo (2004) revealed

Table 2. Growth and yield performance of *A. polytricha* at MR and TC, TNAU, Coimbatore.

S/N	Substrate	DFSR	DFPF	DFFH	I yield	II yield	Total cropping period	Total yield	Bio efficiency (%)
1	Paddy straw	30.0^{e}	40.0^{d}	45.3^{cd}	70.0^{c}	65.0^{b}	56.6^{dc}	135.0	54.00
2	Paddy straw+wheat bran (3:1)	21.3^{g}	31.3^{f}	35.6^{e}	77.3^{a}	70.3^{a}	50.6^{e}	147.6	59.04
3	Paddy straw+rice bran (3:1)	26.0^{f}	37.0^{e}	42.6^{de}	73.6^{b}	66.6^{b}	55.0^{ed}	140.2	56.08
4	Paddy straw+saw dust (3:1)	41.0^{b}	47.6^{b}	52.3^{ab}	60.0^{e}	52.6^{e}	64.6^{b}	112.6	45.04
5	Paddy straw+wheat bran (1:1)	33.6^{d}	43.0^{c}	48.3^{c}	66.6^{d}	59.0^{c}	59.3^{c}	125.6	50.24
6	Paddy straw+rice bran (1:1)	36.6^{c}	44.3^{c}	50.6^{b}	62.0^{e}	55.3^{d}	63.6^{b}	117.3	46.92
7	Paddy straw+saw dust (1:1)	46.3^{a}	51.0^{a}	55.0^{a}	57.0^{f}	50.3^{e}	69.3^{a}	107.3	42.92
	CD (P=0.05)	1.12	2.96	2.80	2.72	2.38	3.43		

Mean of three replications: DFSR - Days for spawn run; DFPF - Days for pinhead formation; DFFH - Days for first harvest.

Table 3. Growth and yield performance of *A. polytricha* at Vijaya Mushrooms, Coimbatore (North).

S/N	Substrate	DFSR	DFPF	DFFH	I yield	II yield	Total cropping period	Total yield	Bio efficiency (%)
1	Paddy straw	30.0^{bc}	37.3^{c}	41.3^{b}	56.0^{c}	47.3^{c}	55.0^{b}	103.3	41.32
2	Paddy straw+wheat bran (3:1)	23.0^{c}	33.0^{f}	33.3^{c}	75.0^{a}	72.0^{a}	47.3^{d}	147.0	58.80
3	Paddy straw+rice bran (3:1)	27.3^{d}	36.0^{e}	35.6^{c}	72.0^{b}	60.0^{b}	49.0^{cd}	132.0	52.80
4	Paddy straw+saw dust (3:1)	30.0^{c}	40.3^{cb}	40.0^{b}	55.3^{c}	46.0^{c}	54.3^{b}	101.3	40.52
5	Paddy straw+wheat bran (1:1)	21.6^{b}	37.6^{c}	41.0^{b}	50.6^{d}	45.0^{c}	53.0^{bc}	95.6	38.24
6	Paddy straw+rice bran (1:1)	35.0^{a}	41.3^{ba}	46.0^{a}	46.6^{d}	40.3^{d}	62.6^{a}	86.9	34.76
7	Paddy straw+saw dust (1:1)	35.0^{a}	43.6^{a}	46.6^{a}	43.3^{d}	36.0^{e}	61.3^{a}	79.3	31.72
	CD (P=0.05)	3.00	2.86	3.19	4.04	2.78	4.09		

Mean of three replications: DFSR - Days for spawn run; DFPF - Days for pinhead formation; DFFH - Days for first harvest.

Table 4. Growth and yield performance of *A. polytricha* at Maha Mushrooms, Coimbatore (south).

S/N	Substrate	DFSR	DFPF	DFFH	I yield	II yield	Total cropping period	Total yield	Bio efficiency (%)
1	Paddy straw	30.0^{d}	35.6^{c}	47.0^{d}	40.0^{e}	36.6^{e}	63.0^{c}	76.6	30.64
2	Paddy straw+wheat bran (3:1)	24.3^{e}	32.3^{d}	33.6^{f}	67.0^{a}	63.0^{a}	47.3^{e}	130.0	52.00
3	Paddy straw+rice bran (3:1)	28.0^{d}	33.3^{bc}	42.6^{e}	60.6^{b}	58.0^{b}	58.0^{d}	118.6	47.44
4	Paddy straw+saw dust (3:1)	35.0^{c}	40.0^{b}	53.0^{c}	57.0^{c}	50.0^{c}	69.3^{b}	107.0	42.80
5	Paddy straw+wheat bran (1:1)	39.6^{b}	41.0^{b}	57.3^{b}	47.0^{d}	43.0^{d}	73.0^{b}	90.6	36.24

Table 4. Cont'd.

S/N	Substrate	DFSR	DFPF	DFFH	I yield	II yield	Total cropping period	Total yield	Bio efficiency (%)
6	Paddy straw+rice bran (1:1)	42.3^{ab}	48.0^{a}	58.6^{ab}	44.2^{e}	40.0^{d}	75.6^{b}	84.6	33.94
7	Paddy straw+saw dust (1:1)	44.6^{a}	46.6^{a}	61.0^{a}	38.0^{e}	38.0^{e}	77.0^{a}	74.3	29.72
	CD (P=0.05)	3.33	2.83	3.43	3.37	3.24	3.97		

Mean of three replications: DFSR - Days for spawn run; DFPF - Days for pinhead formation; DFFH - Days for first harvest.

that wheat straw when supplemented with 10% wheat bran was the most suitable as it provided 93% bioefficiency in mushroom production. This finding supported the result from the present study that paddy straw + wheat bran beds showed faster growth and higher yield of *A. polytricha.* Lin et al. (1993) reported that dead branches, fallen leaves and pruning wastes from tea plants were suitable for cultivation of *A. polytricha.*

Conclusion

The mushroom strains are highly suitable at different substrates under simple low cost substrates which are presently needed for commercial cultivation. The new medicinal mushroom *A. polytricha* has greater scope in commercial exploitation in the globe. The simple production technique comes with considerable yield. As a new introduction to the mushroom world, it is no doubt that the country has greater prospects with wide potentiality to exploit this mushroom.

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