Madras Agric. J., 103 (1-3): 35-40, March 2016

Detection of Variability among the Strains of *Volvariella volvacea* (Bull. Ex Fr.) Sing. and *Volvariella bombycina* (Schaeff.) Sing. Using RAPD analysis

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A total of 9 strains consisting of both *V. volvacea* (5 cultivated strains *viz.*, CBE TNAU 1401 to 1405; 2 wild strains *viz.*, CBE TNAU 1505 and 1516) and *V. bombycina* (one cultivated strain CBE TNAU 1406 and one wild strain CBE TNAU 1504) were evaluated to ascertain the variation in their morphological, cultural and molecular characters. Among strains tested, CBE TNAU 1505 was the best strain, which showed earliness in spawn run, more biomass production in unit time and more chlamydospores production with high density. In the trials conducted with partially decomposed paddy straw, the highest yield of 1041.2 g per 5 kg of substrate with an average biological efficiency of 20.8 per cent was recorded with CBE TNAU 1505. Molecular detection of Volvariella strains through 5.8S rDNA regions showed that ITS primers had amplified a product size between 700 and 740 bp. RAPD - PCR phylogenetic analyses revealed 2 major clusters. *V. volvacea* strains showed approximately 13 per cent genetic similarities with the strains of *V. bombycina*.

Key words: *V. volvacea*, *V. bombycina*, Chlamydospore, Morphology, Strains, ITS and RAPD

Indian mushroom industry is witnessing a fabulous change in recent years with respect to the types and strains cultivated (Krishnamoorthy, 2014). Volvariella belonging to the family Pluteacea (Kolt and Pouz) of Phylum Basidiomycotina ranks sixth amongst the cultivated mushrooms accounting to about 5 per cent of the total world mushroom production. China has produced 3, 30,000 tons accounting for more than 80 per cent of global production (Bao *et al.*, 2013). *Volvariella volvaceae* (Bull. Ex Fr.) Sing. is known as paddy straw mushroom or Chinese mushroom that grows in tropical and sub-tropical regions. Although *V. volvacea* has been cultivated for 300 years, multiple problems associated with the practice have greatly restricted the development of paddy straw mushroom industry. In India, Krishnamoorthy *et al*. (2005) developed a new conscionable approach by cultivating paddy straw mushroom as an intercrop in maize fields with an average biological efficiency of about 8.75 per cent and explored the possibility of introducing this mushroom as an intercrop in high temperature and high humid agro eco system. Although, Volvariella is an aggressive colonizer of any cellulosic substrate, its competitive saprophytic ability, hydrolytic enzyme production potential is very poor. Cellulases play a critical role during substrate colonization while, laccase dominates during sporophore development. These enzymes were found to be expressed differently with different strains of *V. volvacea* (Ahlawat *et al.*, 2005). However, variations occur in the isolates of same strains and needs efficient screening to obtain the isolates with desired attributes. Keeping this background, the present study aimed to explore the best strain of Volvariella and to detect the variation in their morphological growth characteristics, yield potential and genetic relatedness using RAPD.

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Materials and Methods

All experiments were conducted at Mushroom Research and Training Centre, Department of Plant Pathology, Coimbatore during 2014-15.

Collection of cultures

In the attempt to conduct the experiment, 6 strains of paddy straw mushroom fungus (*Volvariella volvacea*) *viz.*, CBE TNAU 1401 to 1405 and *V. bombycina* strain CBE TNAU 1406 used in the study were collected from the germplasm bank of Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore, India. The wild strains namely, CBE TNAU 1504, 1505 and 1516 used were isolated from various habitats of Tamil Nadu such as dead wood of *Ceiba pentandra* (Theni), compost of paddy straw (Perundurai) and paddy straw waste (Coimbatore).

In vitro testing of growth and biomass production

The strains were maintained on Potato Dextrose Agar (PDA) medium in Petri dishes and 100 ml of Potato dextrose broth in 250 ml conical flasks. The media was sterilized at 121°C for 30 min in an

autoclave. Each flask and Petridish was inoculated with 9 mm mycelial discs of strains and incubated at 32 + 2°C for seven days. The radial mycelial growth, colony characters, formation of aerial hyphae and the intensity of chlamydospores were recorded for every 24 h. In order to maintain the vigour, fresh isolations were made from the fruiting bodies every time after 2 to 3 subcultures. For this purpose the strains were propagated in straw spawn and grown on paddy straw following the method suggested by Thomas *et al.* (1943). To measure the quantity of biomass produced, the mycelial mats that had grown on potato dextrose broth were filtered separately through pre-weighed Whatman No.1 filter paper, oven dried at 55 to 60°C for constant weight and the final weight was determined by assessing the weight difference. Aerial mycelial growth, colony characters, days taken for chlamydospores formation and the density were recorded by visible observation. Micrometric observation on the diameter of hyphae and chlamydospore were observed with the help of image analyzer (N-400T, Optika, Italy).

Evaluation of yield potential

In order to evaluate the yield potential, circular compact bed method was followed by using paddy straw based spawn (Sangeetha, 2002) with well performing 6 strains *i.e*. CBE TNAU 1401, 1402, 1404, 1406, 1505 and 1516. Yield potential of the selected strains, were confirmed by conducting two trials *i.e.,* trial 1 during September - October, 2014 and trial 2 during December – January, 2015. Steam sterilized paddy straw twists of 2.5 m length and 5 to 8 cm diameter; each twist weighing 1.25 kg was used for bed preparation. The twists were presoaked in cold water for about 24 h and steam sterilized at 1.46 kg / cm^2 for 1 h. Later, they were shade dried to get 65 to 75 per cent moisture. The twists were compactly placed clockwise in a circular fashion as close as possible on a wooden plank to make the first layer. Straw spawn was placed at the periphery of the first layer of the bed and 20 g of autoclaved horse gram powder was sprinkled over spawn. Second layer was formed over the first layer following the same procedure but the twist was placed compactly in anticlock wise direction. Similarly, the third and fourth layers of bed were formed. The size of the bed measured 30 cm diameter and 20 cm height. Total weight of each bed was 5 kg on dry weight basis. The perfectly prepared bed was pressed tightly and placed in a poly house for cropping at 32 to 35°C and relative humidity of 80 to 85 per cent. Based on the yield data, biological efficiency was calculated based on the following formula: BE (%) = Fresh weight of the mushroom yield in kg / quantity of dry substrate used x 100.

Extraction of total genomic DNA

Total genomic DNA was extracted following the method suggested by Liu *et al*. (2000). The mycelial mats of the 9 Volvariella strains were harvested by filtration through double layers of country filter paper and dried. One g of the dried mycelial mat was ground to fine powder using liquid nitrogen. Powdered mycelia were vortexed in pre-cooled CTAB buffer (N-cetyl-N, N, N trimethyl ammonium bromide 2 g, 0.1 M Tris Hcl (pH: 8.0), 1.4 M NaCl, 0.5 M EDTA (pH: 8.0), 1 g polyvinyl pyrolidone (PVP), 1 ml mercaptoethanol and 1 g of sodium sulphite and incubated at 65°C for 30 min followed by the addition of 750 µl of chloroform and isoamylalcohol (24:1 v/v). The contents were gently mixed to form an emulsion by inverting the tube for 4-5 times and then centrifuged at 10,000 rpm for 10 min. The upper aqueous phase (300 µl) was taken without disturbing the inter phase in a fresh 1.5 ml microfuge tube and added with half the volume of 5 M NaCl and two times the volume of ice cold ethanol. The contents were mixed well and incubated at -20°C for overnight. The contents were centrifuged at 13,000 rpm at 4°C for 10 min; the ethanol fraction was decanted and the DNA pellet was air dried. Later, the pellet was resuspended in 50 µl of Tris-EDTA buffer (10 mM Tris-HCl and 1mM EDTA, pH 8.0). The genomic DNA was checked by using 0.8 per cent agarose gel electrophoresis and stored at -20°C for further use.

Amplification of 5.8S rDNA

The Polymerase Chain Reaction (PCR) amplification was performed by using the primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC -3') to amplify the ITS region along with 5.8S rDNA. PCR was undertaken in 30 µl reaction mixture containing 3 µl of template DNA (50 ng), 15 µl of master mix (Bangalore Genei, Pvt. Ltd., Bangalore, India), 3 µl each of ITS primers (0.01 mM) and 6 µl of nuclease free water (Fermentas, USA). The reactions were performed in Eppendorf gradient S Master cycler (Eppendorf, Hamburg, Germany). Cycling conditions were 94°C for 5 min followed by 36 cycles at 95°C for 1 minute, 50°C for 30 seconds, 72°C for 80 seconds and final extension step at 72°C for 10 min with lid heating option at 104°C. The PCR amplified products (10 µl) were electrophoretically separated by running the agarose gel prepared in 1x Tris-acetate-EDTA (TAE) buffer (1.2 g in 100 ml of buffer) at 80 V for 1h. Staining was done with ethidium bromide and the gel was visualized and photographed using Bio-rad imaging system. Comparison of DNA amplicon length with standard molecular weight marker (100 bp) was done and conclusions were made.

RAPD analysis

Multilocus genotyping was performed by RAPD using 9 decamer primers, OPA 01(5'-CAGGCCCTTC-3'), OPA 03 (5'-AGTCAGCCAC-3'), OPA 5 (5'-AGG GGTCTTG-3'), OPA 07 (5'-GAAACGGGTG-3'), OPA 09 (5'-GGGTAACGCC-3'), OPA 18 (5'-AGGTGACCGT-3'), OPB 03 (5'-CATCCCCCTG-3'), OPB 11 (5'-GTAGACCCGT-3') and OPB 15 (5'-GGAGGGTGTT-3'). Amplification was performed in 30 µl reaction mixture, containing 6 µl primer (50 pM), 15 µl of master mix (Bangalore Genei, Pvt. Ltd.,

Bangalore, India), 3 µl template DNA (50 ng) and 6 µl of nuclease free water (Fermentas, USA). RAPD-PCR amplifications were performed in Eppendorf gradient S Master cycler (Eppendorf, Hamburg, Germany) with initial denaturation at 94°C for three min followed by 40 cycles of 94°C for forty seconds, 40°C for forty seconds and 72°C for one min and final elongation at 72°C for 10 min with lid heating option at 104°C. The agarose gel was prepared as mentioned above and electrophoresis was performed by using standard molecular weight marker (1 Kb). The band profile of each gel was scored as '1' for the presence and '0' for absence of bands and combined binary data matrix for all the markers was constructed. The data matrix was entered in the numerical taxonomy and multivariate analysis system package NTSYS-pc programme version 2.2 (Numerical Taxonomy System, Applied Biostatistics Inc, New York) to estimate similarity indices using SIMQUAL route to obtain Jaccard's similarity coefficients (Jaccards, 1908). The similarity coefficients were used to construct the dendrogram employing the Unweighted Paired Group Method using Arithmetic averages (UPGMA) and the SAHN (Sequential Agglomerative Hierarchical and Nested Clustering) using NTSYS software. Four replications were maintained for each strain in every experiment. The statistical analyses of all the experiments conducted were laid out based on Completely Randomized Block Design (CRBD) (Gomez and Gomez, 1984). Statistical software AGRES was used for the analyses of the data. In case of zero values the data was log transformed (X+0.5) before statistical analysis.

Results and Discussion

Selection of Volvariella strains based on morphological characters and yield potential

The nine strains of Volvariella, varied in their morphological characters such as aerial mycelial growth, colony characters, earliness of chlamydospores production and their density on PDA

Table 1. Selection of superior strains of *Volvariella* spp based on morphological characters

Strain	Radial growth in mm (6 DAI)*	DTTCPP*	Aerial hyphae*	Colony morphology	Bio mass in g 15 DAI*	Performance in broth	Chlamydospores density*	DTFCP*	Micrometric observations#	
									Hyphal dia (µm)	Chlamydo spore dia (µm)
CBETNAU 1401	90.0a	5.5cd	+++	Depressed in centre and raised in margins	0.9c	Floating on surface	++	16.8e (4.1)	4.8	40.0
CBETNAU 1402	90.0a	5.2bc	++++	Thick Cottony	1.1b	Floating on surface	+++	14.5c (3.8)	6.4	44.4
CBETNAU 1403	52.1c	11.4h	++	Fried egg like	0.7ef	Floating on surface	-	NP	3.8	-
CBETNAU 1404	62.0bc	9.5f	+++	Fried egg like in centre and cottony towards margins	0.8d	Floating on surface	+	16.6e (4.1)	5.2	43.7
CBETNAU 1405	65.7bc	8.6g	-	Depressed and uniform	0.7de	Floating on surface	-	NP	3.3	-
CBETNAU 1406	90.0a	4.9ab	+	Slightly yellowish and raised in centre	0.6g	Submerged	++++	7.3a (2.8)	5.3	22.5
CBETNAU 1505	90.0a	4.7a	++++	Depressed in centre and raised in margins	1.2a	Floating on surface	+++	13.8c (3.7)	7.3	24.2
CBETNAU 1504	81.0a	5.6d	+	Irregular and raised	0.7f	Submerged	++++	8.5b (3.0)	7.1	28.5
CBETNAU 1516	76.5ab	7.3e	+	Greyish and regular	0.8d	Submerged	++	15.5d (4.0)	5.5	28.1
CD (P = 0.05)	15.0	0.3			0.05			0.11		

Aerial hyphae, chlamydospores density "- to ++++" absent to highly densed.
DTTCPP – Days taken to cover 90 mm Petri plate. NP – not produced.
DTFCP – Days taken for chlamydospores production.
*, # Values are mean of 4 and 25 replications. Data in parenthesis were square root transformed values.
Figures in parentheses are log transformed values, Means followed by a common letter are not significantly different at P = 0.05 by one way ANOVA.

medium (Table 1 and Fig.1). The highest radial growth of 90 mm was observed in strain CBE TNAU 1505, which took 4.7 d to complete full plate, followed by CBE TNAU 1406, 1402 and 1401 (4.9 d, 5.2 d and 5.5 d, respectively). The strain CBE TNAU 1403 took longer time for proliferation and was found to be less virulent (52.1 mm) and took 11.4 d to complete full plate. The production of chlamydospores was first observed in CBE TNAU 1406 in 7.3 d, followed by CBE TNAU 1504 in 8.5 d; while, CBE TNAU 1401 took 16.8 d. No chlamydospore production was observed in CBE TNAU 1403 and 1405. The strain CBE TNAU

Table 2. Yield performance of different isolates of *Volvariella* spp

Strain	Colour of egg	Trail 1 (September – October, 2014) *							Trail 2 (December, 2014 – January,2015) *						
		DFSR	DFPF	DFFH	No. of eggs harvested	Avg. weight of egg (g)	Avg. yield (g)	BE (%)	DFSR	DFPF	DFFH	No. of eggs harvested	Avg. weight of egg (g)	Avg. yield (g)	BE (%)
CBE TNAU 1401	Greyish	6.5	7.1^{bc} (2.7)	11.5^{b} (3.4)	39.1^{b} (6.2)	22.3^{ab} (4.7)	874.0^{b} (29.5)	17.4^{c} (25.0)	7.2	8.8	13.2^{d}	36.9^{c}	20.3^{d}	751.8^{c} (27.4)	15.0^{c} (22.8)
CBE TNAU 1402	Greyish	5.7	6.8^{ab} (2.7)	10.8^{a} (3.3)	43.3^{a} (6.6)	22.8^{a} (4.8)	991.1^{a} (31.4)	19.8^{b} (26.7)	6.2	7.5	12.1^{b}	40.3^{b}	21.6^{c}	876.3^{b} (29.6)	17.5^{b} (24.7)
CBE TNAU 1404	Greyish	7.1	7.8^{d} (2.8)	12.7^{c} (3.6)	31.4^{d} (5.6)	21.4^{b} (4.6)	674.6^{d} (25.9)	13.4^{e} (21.9)	7.7	9.1	14^{e}	24.7^{e}	24.5^{a}	608.4^{d} (24.6)	12.1^{d} (20.3)
CBE TNAU 1406	White	10.4	NP	NP	NP	NP	NP	NP	6.7	10.5	11.2a	2f	16.3e	32.6^{e} (5.7)	0.6^{e} (4.6)
CBE TNAU 1505	Greyish	5.2	6.6^{a} (2.6)	10.4^{a} (3.3)	44.9^{a} (6.7)	23.1^{a} (4.8)	1041.2^{a} (32.2)	20.8^{a} (27.4)	5.8	7.16	11.1^{a}	42.7^{a}	22.0^{bc}	940.7^{a} (30.6)	18.8^{a} (25.6)
CBE TNAU 1516	Greyish	6.8	7.3^{c} (2.7)	12.0^{b} (3.5)	36.1^{c} (6.0)	22.0^{ab} (4.7)	797.8^{c} (28.2)	15.9^{d} (23.9)	7.1	8.3	12.8^{c}	33.8^{d}	22.6^{b}	767.9^{c} (27.7)	15.3^{c} (23.0)
CD (P =0.05)		NS	0.08	0.09	0.1	0.1	0.8	0.4	NS	NS	0.3	1.0	0.8	0.7	0.4

DFSR - Days taken for spawn run.

DFPF - Days taken for pinhead formation.

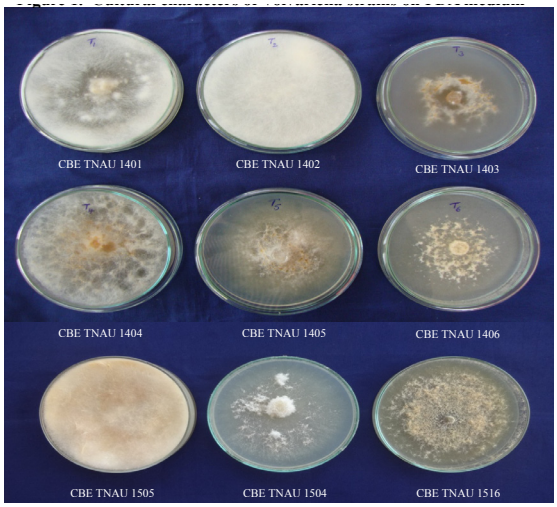
DFFH - Days taken for first harvest.

BE - Biological efficiency (The data in parenthesis are arcsine transformed values).

*Values are mean of four replications. Means followed by a common letter are not significantly different at P = 0.05 by one way ANOVA. Data in parenthesis were square root transformed values.

1505 produced significantly more biomass (1.1 g) on 15 DAI under *in vitro.* This was followed by CBE TNAU 1402 and 1401 (1.0 and 0.9 g), whereas CBE TNAU 1406 produced less biomass of only 0.6 g. The

Fig. 1. Cultural characters of Volvariella strains on PDA medium



mycelial mats had grown very well over the surface of the broth except in case of CBE TNAU 1406, 1504 and 1516 where the mats were partially submerged (Table 1). Days taken for chlamydospore production and the density were recorded by the presence of pink coloured colonies in the Petriplate however, the procedure to accurately measure the chlamydospore density based on optical density is lacking and future studies in this context are warranted. Chang *et al*, (1981) corroborated that monosporous isolates of paddy straw mushroom varied in their growth rate, abundance of mycelia, aerial hyphae and presence of chlamydospores. They divided isolates of paddy straw mushroom into five different groups ('NN', 'N', 'AN', 'A' and 'AA') based on their morphological characteristics.

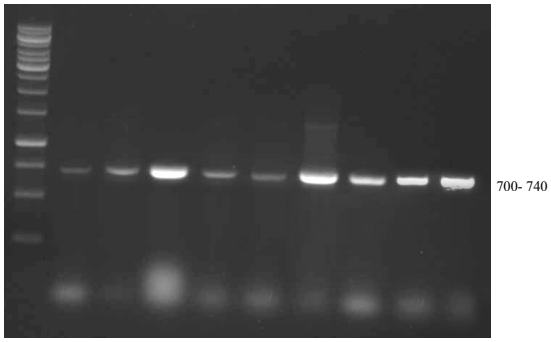
In the present investigation, the yield potential of six strains which performed better under in vitro studies *i.e.*, CBE TNAU 1401, 1402, 1404, 1406, 1505 and 1516 was evaluated by conducting two independent trials (one in September to October, 2014 and the other in December 2014 to January,

Fig. 2. Fruiting bodies of Volvariella strains grown on paddy straw

2015) on partially decomposed paddy straw. Among the strains evaluated during trial 1, CBE TNAU 1505 and CBE TNAU 1402 performed significantly superior and found to be on par by producing the maximum yields and biological efficiencies of 1041.2 g, 20.8 per cent and 991.1 g, 19.8 per cent per 5 kg of substrate, respectively. This was followed by CBE TNAU 1401 (874.0 g and 17.4 per cent) and CBE TNAU 1516

(797.8 g and 15.9 per cent). Minimum yield and biological efficiency was recorded with CBE TNAU 1404 (674.6 g and 13.4 per cent). CBE TNAU 1406 strain was significantly different from all Fig.3.

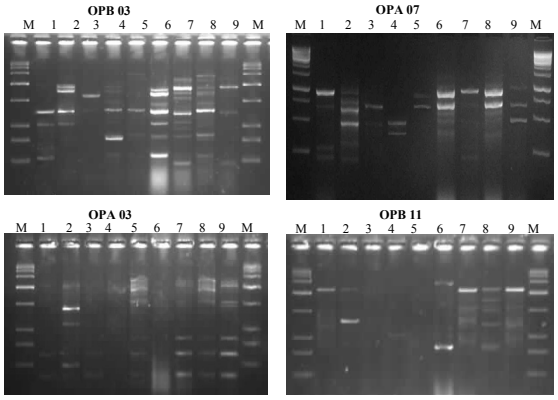
Fig. 3. PCR amplification of ITS regions of *Volvariella* strains



M - 1 kb ladder 4 - CBE TNAU 15058 - CBE TNAU 1504
1 - CBE TNAU 1401 5 - CBE TNAU 14049 - CBE TNAU 1516
2 - CBE TNAU 1402 6 - CBE TNAU 1405
3 - CBE TNAU 1403 7 - CBE TNAU 1406

PCR amplification of ITS regions of Volvariella strains the strains in all parameters and recorded no yield. In the second trial, all the strains except CBE TNAU 1406 were found to be on par in terms of yield. CBE TNAU 1406 produced minimum yield of only 32.6 g per 5 kg substrate (Table 2 and Fig.2).

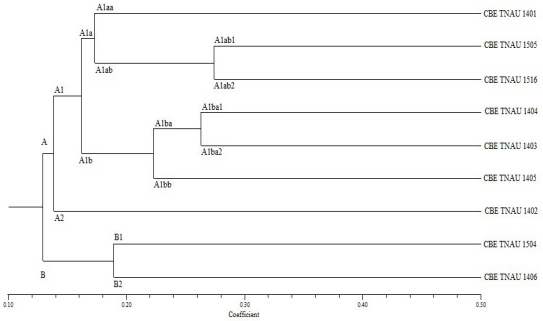
Fig. 4 a. RAPD profile of Volvariella strains amplified with different primers



M - 1 kb ladder
1 - CBE TNAU 1401
2 - CBE TNAU 1402
3 - CBE TNAU 1403
4 - CBE TNAU 1505
5 - CBE TNAU 1404
6 - CBE TNAU 1405
7 - CBE TNAU 1406
8 - CBE TNAU 1504
9 - CBE TNAU 1516

The data presented in Table 1 and 2 revealed that the strain, CBE TNAU 1505 had exhibited the highest growth with plenty of aerial hyphae, chlamydospores density and yield performance with more biological efficiency followed by cultivated strain CBE TNAU 1402. Ahlawat *et al*. (2010) disclosed that there was no relationship between chlamydospores formation, rate of mycelial growth and yield. In a separate study, Ahlawat *et al*. (2014) also screened ten strains of *V. volvacea*, among which the fastest growing strain OSM-1 exhibited the highest activity of exoglucanase, low level of endoglucanase and increased levels of β-glucosidase and xylanase. This perspicuously indicates that strain CBE TNAU 1505 may have exhibited more activity of hydrolytic enzymes on comparison to other strains.

Fig. 4 b. Dendrogram of molecular phylogenetic relationship of Volvariella strains



V. volvacea strains CBE TNAU 1401, 1402, 1404, 1505 and 1516 are adapted to wide varied temperatures between 29 and 36°C and produced better yields; whereas, *V. bombycina* strain produced a rudimentary fruiting body at the temperature between 26 and 31°C. According to Datta and Chakravarty (2002) *V. volvacea* was found to utilize cellulose and hemicellulose throughout spawn run and was unable to utilize lignin at any stage due to lack of lignolytic system. Substrates preference by *V. volvacea* and *V. bombycina*, respectively could be well understood from the fact that *V. volvacea* collected from partially fermented straw and *V. bombycina* from the dead wood of Ceiba pentandra. In confirmity with Singh (2014), *V. bombycina* requires the temperature of 26 to 30°C for mycelial growth and fruiting. This lucidly indicates that low temperature and lignicolous substrate are suitable for extensive growth and fruiting of *V. bombycina.*

Molecular variation and genetic relatedness of Volvariella strains using RAPD

PCR amplification of 5.8S rDNA region was performed with the nine strains of *Volvariella* sp. by using ITS 1 and ITS 4 primers. The DNA of nine strains amplified approximately between 700-740 bp (Figure 3). 2 parent and 15 monosporous isolates of *V. volvacea* amplified approximately 720 bp product in all the strains (Ahlawat *et al*., 2010). Though the amplification was observed at 700-740 bp, comparing the scrupulous results of earlier workers it is concluded that amplification of ITS 1 and 4 regions alone will not provide cent per cent agreement to confirm the phylogenetic relationship between the tested strains.

To study the molecular variation and genetic relatedness among the nine strains, RAPD was performed by using 9 random decamer primers, out of which 4 primers produced no bands in most of the strains. The DNA fragments showed amplification with different molecular weights up to 2000 bp (Figure 4a). Reproducibility of the amplification pattern was confirmed in two independent experiments. Based on the analysis made from RAPD-PCR banding patterns, all the nine strains of Volvariella were grouped into 2 major clusters A and B (Figure 4b) with *V. volvacea* strains CBE TNAU 1401, 1402, 1404, 1405, 1505, 1403 and 1516 in cluster A; V.

bombycina strains CBE TNAU 1504 and 1406 in cluster B. The cluster A is again divided into 2 sub clusters A1 and A2. Sub cluster A2 consists of CBE TNAU 1402, which was approximately 13.8 per cent similar with the strains of A1. Cluster B consists of *V. bombycina* strains CBE TNAU 1504 and CBE TNAU 1406 at 19 per cent similarity, which were approximately 13 per cent similar with that of strains in cluster A. Analysis of the RAPD showed minimum and maximum per cent similarities among the strains of *Volvariella,* which were in the range between 13 and 27.5 per cent, respectively. Whereas, Ahlawat *et al.* (2010) purported intra specific variation of 60 per cent within the 2 parent strains and their single spore isolates of *V. volvacea*. Whole genome of *V. volvacea* with a total genome size of 35.7 Mb was sequenced by Bao *et al*. (2013). This provided explicit understanding of molecular levels involved in sexual reproduction mechanism, degradation of compost and the sensitivity to low temperatures by *V. volvacea*. Commercial exploitation and application of this genome information may be used to improve the chlamydospores production, yield and shelf life of *Volvariella* sp by regulating the responsible genes. The pattern of distribution of genotypes into two broad clusters at random indicated that geographical and genetic diversity were not related. This further suggested that forces other than geographical origin such as genetic drift, natural and artificial selection, exchange of cultivars might have played an important role in the evolution of diversity of tested strains. Variations in environment could also be responsible for such diversity. The RAPD data presented here confirmed that, the genetic similarity among the strains exists and the level of similarity is medium. High yielding and chlamydospores producing wild strain of *V. volvacea* CBE TNAU 1505 was found to be genetically 27.5 per cent similar to wild strain CBE TNAU 1516. Varied conscientious reports indicate that the DNA fingerprints showed polymorphism between strains of *V. volvacea* and *V. bombycina.* Systematic investigations in this context may give lead for exploration of elite Volvariella strains.

Conclusion

From this concerted study, it was straight laced that Volvariella strains showing variation in morphological and cultural characteristics also showed variation in their RAPD profiles revealed through phylogenetic analysis. The strain specific RAPD bands of high yielding Volvariella strains could be utilized to identify the phylogenetic belongingness.

Acknowledgement

The authors express their gratitude to Department of Plant Pathology, TNAU, Coimbatore and ICAR-AICRP on mushroom for the valuable support rendered during the course of investigation.

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Received after revision: February 12, 2016; Accepted: March 28, 2016