Short communication

Variability of paddy straw mushroom collections from southern Kerala

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Abstract

The occurrence of the underutilized as well as highly tasty paddy straw mushroom is reported to be more in Kerala. A survey was conducted to explore the native isolates of *Volvariella* sp. and to study their morphological as well as microscopic characters. Thirteen paddy straw mushroom collections were obtained from two districts of south Kerala. During the survey, habit, habitat, substrate, minimum and maximum temperature of the location, relative humidity and rainfall data were recorded and compared. Morphological and microscopical characteristics of the collections were studied in detail and identified up to species level. *Volvariella volvacea* predominated among the collections. The collections with superior morphological and microscopical characters in this study can be effectively utilized for cultivation purpose.

Keywords : Paddy straw mushroom, *V. gliocephala, V. hypopithys, Volvariella* sp., *V. volvacea*

Paddy straw mushroom (*Volvariella* sp.) is the third most popular mushroom in the world (Graham et al., 2004) and ranks sixth among the most cultivated mushrooms (Aida et al., 2009). Taxonomic studies involving this genus have been taking place all over the world including India in the past decade (Dwivedi et al., 2012; Senthilarasu et al., 2015). As a result many species were identified and were added to the floral diversity of India. The recent additions include *V. earlei* (Murrill) Vizzini and *V. gloiocephalus* (DC.) Justo (Amandeep et al., 2015), *V. speciosa* (Fr.) Singer (Venkatachalapathi and Paulsamy, 2016). According to the latest records, more than 15 species of the genus *Volvariella* has been reported belonging to 28 taxa have been reported from India while 91 taxa are registered till date in Mycobank (Kaur, 2017).

The cultivation aspects and nutritional studies regarding this mushroom are gaining momentum nowadays (Wang et al., 2017). But the productivity of this mushroom is found highly variable even though suitable climatic conditions prevail in India and Kerala. As an alternative solution, the native isolates of the mushroom from different locations can be studied, and if found superior in the favourable characters, can be made use of for cultivation in that location. In this context, this study was performed to compare the morphological and microscopical characters of locally available isolates of *Volvariella* sp. and to interpret their suitability to cultivation in the area.

During South-West and North-East monsoon seasons of 2015 - 2016 surveys were conducted at different locations of Thiruvananthapuram and Kollam districts for collection of native flora of paddy straw mushroom growing under natural condition. Native isolates of *Volvariella* sp. were collected from various locations *viz.* Vellayani, Arippa, Neeramankara, Poonkulam, Palode, Anchal and Bharathipuram. Observations on habit, habitat,

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temperature, relative humidity on the day of collection and rainfall on the preceeding day of collection were noted. Observations regarding the weight, colour and texture of sporocarp at egg stage/ immature stage; pileus diameter, margin; stipe length, thickness, consistency, colour; length of volva; gill arrangement, consistency, colour, texture, margin, gill number per centimetre were made from the collected native isolates. Microscopical studies on spore, basidia and cystidia were made using stage and ocular micrometers and Zeiss/ Axic Lab stereo microscope by mounting sections of gill in Congo red dye (Justo and Castro, 2010) and observations were taken. Spore prints of the collections were also made and studied.

The morphological and microscopical characters were compared with the available keys and identification of the collections were done according to Moser (1983), Purkayastha and Chandra (1985) and Singer (1986).

Using standard tissue culture technique, cultures were developed from the bulbous base region of the collected mushrooms.

The isolate with superior morphological and microscopical characters was molecularly characterized using ITS sequencing and the blast sequence was analysed in ClustalX2 software. Dendrogram was also constructed using TreeView software showing the phylogenetic relationship.

From the survey, thirteen collections were obtained from Vellayani, Neeramankara, Poonkulam and Palode regions of Thiruvananthapuram district and from Arippa, Anchal and Bharathipuram regions of Kollam district. The collections were mainly obtained during May, January, August and November months. The majority of the collections were obtained in November which was a post-monsoon month at a lower atmospheric temperature and higher relative humidity. Only two collections were obtained during pre-monsoon period. Minimum temperature ranged from 23.7 to 25.8°C, and maximum temperature ranged from 31.3 to 35.6°C, and the relative humidity on the day of collection ranged from 85 to 95%. Rainfall on the previous day of collection was recorded as it accelerated mushroom emergence and it was found varying among the collections. All the collections were from terrestrial habitat and the details of the collections are described in Table 1. Morphological studies on collections of paddy straw mushroom were carried out recording dimensions and features of sporocarps, stipe, gills, and volva.

It was noted that Poonkulam isolate (V6) had the maximum sporocarp weight (18.8 g) compared to the remaining isolates. All the isolates had sporocarps weighing more than 10 g. V9 have an average sporocarp weight of 17.1 g and it occurred in clusters of 13 sporocarps. Pileus characters of all the isolates were studied during their immature and mature stages. All the isolates had polished texture during their immature stage and had a smooth texture at maturity. Generally pileus was sub-umbonate with regular margin having free, smooth, pale salmon to moderate brown gills and central, cylindrical stipe with bulbous base encased in a thick white to grayish sac-like volva. The diameter of pileus was found to be larger (14.8 cm) for V6 (Table 2). Cap colour at periphery region, middle region and top region of all the isolates were observed. Periphery cap colour of all the isolates was light yellow. At middle region it was light yellow for V1, V2 and V3, pale yellowish grey for V6 and light olive brown for the remaining isolates. The top of pileus was moderate olive brown or moderate brown except for V6 with pale yellowish grey colour. The pileus margin of almost all the isolates was regular and entire (Table 2).

Stipe length of all the isolates were found comparable and ranged from 10 – 15cm. The thickness of stipe was also found similar for all the isolates. The stipe of all the isolates was cylindrical in shape with central type of attachment with pileus and showed fleshy consistency. All the collections had yellowish white coloured stipe with thick,

Table 1. Details of native isolates of *Volvariella* under natural conditions

Collection No.	Location	Habit	Latitude and Longitude of Habit	Substrate	Min. Temperature (°C)	Max. Temperature (°C)	RH (%)	Month and Year of Collection	Weight of Sporocarp (g)	Rainfall (mm)
V1	Vellayani	Solitary	8.4344° N, 76.9917° E	Banana pseudostem waste	24.1	32.4	73/92	May 2015	11.8	1.2
V2	Vellayani	Gregarious (cluster of 9)	8.4290ÚN, 76.9864ÚE	Banana pseudostem base	24.3	35.6	78/95	August 2015	13.6	5.3
V3	Vellayani	Gregarious (cluster of 3)	8.4455ÚN, 76.9917ÚE	Banana pseudostem base	24.3	35.6	78/95	January 2016	12.9	0.4
V4	Arippa	Gregarious (cluster of 5)	8.8513ÚN, 77.0411ÚE	Oilpalm bunch waste	23.7	31.6	91/93	August 2015	13.0	5.7
V5	Neeramankara	Solitary	8.4759ÚN, 76.9707ÚE	Banana pseudostem base	24.4	32.8	77/92	November 2015	16.0	11.0
V6	Poonkulam	Gregarious (cluster of 4)	8.4230ÚN, 76.9715ÚE	Agrowastes	25.8	32.6	75/89	November 2015	18.8	8.8
V7	Palode	Solitary	8.7533ÚN, 77.0262ÚE	Oilpalm bunch waste	24.6	31.3	87/ 97.5	November 2015	15.3	12.6
V8	Palode	Gregarious (cluster of 3)	8.7084ÚN, 77.0290ÚE	Oilpalm bunch waste	24.6	31.3	87/ 97.5	November 2015	12.6	12.6
V9	Palode	Gregarious (cluster of 13)	8.7038ÚN, 77.0289ÚE	Oilpalm bunch waste	24.6	31.3	87/ 97.5	November 2015	17.1	12.6
V10	Anchal	Gregarious (cluster of 2)	8.9300ÚN, 76.9065ÚE	Oilpalm bunch waste	24.6	31.3	87/ 97.5	November 2015	14.1	10.8
V11	Anchal	Gregarious (cluster of 4)	8.9333ÚN, 76.9456ÚE	Oilpalm bunch waste	24.6	31.3	87/ 97.5	November 2015	12.8	10.8
V12	Anchal	Solitary	8.9387ÚN, 76.9479ÚE	Oilpalm bunch waste	24.6	31.3	87/ 97.5	November 2015	14.7	10.8
V13	Bharathipuram	Gregarious (cluster of 2)	8.9294ÚN, 76.9859ÚE	Oilpalm bunch waste	24.6	31.3	87/ 97.5	August 2015	10.4	9.6

Table 2. Pileus characters of native isolates of *Volvariella* spp.

Isolate	Dia (cm)	Colour			Margin
		Outer circle	Middle circle	Inner circle	
V1	8.0	Light yellow	Light yellow	Moderate olive brown	Regular
V2	6.0	Light yellow	Light yellow	Moderate olive brown	Regular
V3	7.2	Light yellow	Light yellow	Moderate olive brown	Regular
V4	6.2	Light yellow	Light olive brown	Moderate olive brown	Irregular
V5	10.1	Light yellow	Light olive brown	Moderate olive brown	Regular
V6	14.8	Light yellow	Pale yellowish grey	Pale yellowish grey	Regular
V7	13.1	Light yellow	Light olive brown	Moderate brown	Irregular
V8	10.5	Light yellow	Light olive brown	Moderate olive brown	Regular
V9	11.7	Light yellow	Light olive brown	Moderate olive brown	Regular
V10	10.8	Light yellow	Light olive brown	Moderate olive brown	Regular
V11	12.6	Light yellow	Light olive brown	Moderate brown	Regular
V12	11.3	Light yellow	Light olive brown	Moderate brown	Regular
V13	8.5	Light yellow	Light olive brown	Moderate olive brown	Regular

Table 3. Stipe characters of native isolates of *Volvariella* spp.

Isolate No.	Length (cm)	Thickness (cm)	Shape	Attachment to pileus	Consistency	Colour	Base	Volva	Volva length(cm)
V1	5.8	0.5-0.9	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.6
V2	6.0	0.6-1.4	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.8
V3	5.9	0.8-2.0	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.9
V4	5.2	0.4-1.5	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.7
V5	11.4	0.4-1.9	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.8
V6	11.0	0.5-1.8	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.9
V7	10.1	0.6-2.1	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.8
V8	10.4	0.4-1.8	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.8
V9	11.1	0.7-1.6	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.7
V10	11.4	0.4-1.7	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.9
V11	10.6	0.5-1.9	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.9
V12	11.8	0.7-2.2	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.8
V13	10.1	0.4-1.3	Cylindrical	Central	Fleshy	Yellowish white	Swollen	Sheathed	1.6

Table 4. Gill characters of native isolates of *Volvariella* spp.

Isolate	Arrangement	Consistency	Colour		Texture	Margin	Gills/cm
			Immature	Mature			
V1	Free	Fleshy	White	Pale Salmon	Smooth	Smooth	10
V2	Free	Fleshy	White	Pale Salmon	Smooth	Smooth	9
V3	Free	Fleshy	White	Pale Salmon	Smooth	Smooth	10
V4	Free	Fleshy	White	Pale Salmon	Smooth	Smooth	12
V5	Free	Fleshy	White	Moderate Brown	Smooth	Smooth	11
V6	Free	Fleshy	Brownish orange	Pale Salmon	Smooth	Smooth	11
V7	Free	Fleshy	Brownish orange	Moderate Brown	Smooth	Smooth	13
V8	Free	Fleshy	Brownish orange	Moderate Brown	Smooth	Smooth	12
V9	Free	Fleshy	Brownish orange	Moderate Brown	Smooth	Smooth	13
V10	Free	Fleshy	Brownish orange	Pale Salmon	Smooth	Smooth	11
V11	Free	Fleshy	Brownish orange	Pale Salmon	Smooth	Smooth	10
V12	Free	Fleshy	Brownish orange	Pale Salmon	Smooth	Smooth	12
V13	Free	Fleshy	Brownish orange	Moderate Brown	Smooth	Smooth	9

swollen, sheathed base called volva. Volva length of all the isolates was recorded and was found comparable (Table 3) ranging from 1.6 – 1.9cm.

Gill characters of all the isolates were studied and it was found almost similar. The fleshy gills of all the isolates were having a smooth texture and smooth margin arranged freely. V1 to V5 had white

Table 5. Microscopic characters of native isolates of *Volvariella* spp.

Isolate	Hyphae Colour	Septation	Spore shape	Spore size (µm)	Spore colour	Spore print	Cystidia shape	Cystidia size(µm)	Basidia shape	Basidia size(µm)
V1	Hyaline	Septate	Ovoid	5-7× 4 - 5	Pink	Pink	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V2	Hyaline	Septate	Ovoid	5-7× 4 - 5	Pink	Pink	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V3	Hyaline	Septate	Ovoid	5-7× 4 - 5	Pink	Pink	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V4	Hyaline	Septate	Ovoid	5-7× 4 - 5	Pink	Pink	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V5	Hyaline	Septate	Ovoid	5-7× 4 - 5	Moderate Brown	Moderate Brown	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V6	Hyaline	Septate	Ovoid	8-9 × 5-6	Pink	Pink	Bottle shaped	20-36 × 5-11	Club shaped	18-21× 6-8
V7	Hyaline	Septate	Ovoid	13-18×8-10	Moderate Brown	Moderate Brown	Bottle shaped	50-80 × 15-40	Club shaped	22-25×10-15
V8	Hyaline	Septate	Ovoid	13-18×8-10	Moderate Brown	Moderate Brown	Bottle shaped	50-80×15-40	Club shaped	22-25×10-15
V9	Hyaline	Septate	Ovoid	13-18×8-10	Moderate Brown	Moderate Brown	Bottle shaped	50-80 ×15-40	Club shaped	22-25×10-15
V10	Hyaline	Septate	Ovoid	5-7× 4 - 5	Pink	Pink	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V11	Hyaline	Septate	Ovoid	5-7× 4 - 5	Pink	Pink	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V12	Hyaline	Septate	Ovoid	5-7× 4 - 5	Pink	Pink	Bottle shaped	23-51 × 11-16	Club shaped	21-24 × 9-11
V13	Hyaline	Septate	Ovoid	5-7× 4 - 5	Moderate Brown	Moderate Brown	Bottle shaped	23-51 × 11-16	Club shaped	21-24× 9-11

coloured gills during immature stage while all the remaining isolates had brownish orange colour. The number of gills present per centimetre length of pileus was studied for all the isolates. It was observed in the range of 9 – 13 (Table 4).

The study of microscopic characters of the isolates revealed that they had septate hyphae without any clamp connection, cheilocystidia were bottle shaped, basidia were club-shaped with four pink/ moderate brown basidiospores. Cheilocystidia, basidia and basidiospores characters are detailed in Table 5. V5, V7, V8, V9 and V13 had moderate brown coloured spores while the remaining isolates had pink coloured spores. Spores were ovoid in shape and size ranged from 5-18 X 4-10 µm (Table 5).

Based on morphological and microscopic characters all the collections were identified. Three isolates obtained from Vellayani (V1, V2, V3), one isolate collected from Arippa (V4), one isolate collected from Neeramankara (V5), three isolates from Anchal (V10, V11, V12) and one isolate collected from Bharathipuram (V13) were identified as *V. volvacea* based on the keys available. The three Palode isolates (V7, V8, V9) were identified as *V. gliocephala*. The Poonkulam isolate (V6) was identified as *V. hypopithys* based on both morphological and molecular data (Table 6). ITS sequencing, followed by comparative nucleotide sequence alignment of the V6 isolate of *Volvariella sp*., with the available data base from NCBI revealed a 97 per cent homology with the *V. hypopithys* isolate from Brazil. The phylogenetic tree was constructed, which grouped the collected isolate and isolates from Italy and Brazil into a single major cluster, signifying a common evolutionary lineage (Fig. 1). *V. volvacea* was obtained from more

Table 6. Identification of the collected isolates of *Volvariella* spp. with their accession numbers

Isolate No.	Location	Accession Number	Morphological Identification
V1	Vellayani	DMRO – 941	*V. volvacea*
V2	Vellayani	DMRO - 952	*V. volvacea*
V3	Vellayani	DMRO - 944	*V. volvacea*
V4	Arippa	DMRO - 849	*V. volvacea*
V5	Neeramankara	DMRO - 942	*V. volvacea*
V6	Poonkulam	DMRO - 943	*V. hypopithys*
V7	Palode	DMRO - 945	*V. gliocephala*
V8	Palode	DMRO - 946	*V. gliocephala*
V9	Palode	DMRO - 947	*V. gliocephala*
V10	Anchal	DMRO - 948	*V. volvacea*
V11	Anchal	DMRO - 949	*V. volvacea*
V12	Anchal	DMRO - 950	*V. volvacea*
V13	Bharathipuram	DMRO - 951	*V. volvacea*

Table 7. Mycelial growth of native isolates of paddy straw mushroom

Isolate	Type of mycelial growth	Dia. of mycelial growth after 3 days (cm)*	Dia. of mycelial growth after 5 days (cm)*
V1	Thick and fluffy	3.73^{e}	7.70^{f}
V2	Thin and sparse	2.80^{h}	7.10^{gh}
V3	Thin and sparse	3.47^{f}	7.50^{f}
V4	Thin and sparse	3.23^{g}	7.23^{g}
V5	Thick and fluffy	4.57^{c}	8.40^{b}
V6	Thick and fluffy	5.20^{a}	8.97^{a}
V7	Thick and fluffy	4.10^{d}	8.33^{bc}
V8	Thick and fluffy	4.70^{c}	8.13^{cde}
V9	Thick and fluffy	4.13^{d}	8.07^{de}
V10	Thick and fluffy	5.07^{ab}	8.27^{bcd}
V11	Thick and fluffy	4.90^{b}	7.97^{e}
V12	Thick and fluffy	3.80^{e}	8.23^{gh}
V13	Thin and sparse	2.00^{i}	6.90^{h}
C.D. (0.05)		0.18	0.22

* average of 3 replications

•Means followed by similar superscripts are not significantly different at 5 % level

locations which indicated its widespread occurrence in south Kerala region. All the cultures isolated from the mushroom obtained during the survey were purified and deposited in Directorate of Mushroom Research, Solan with the accession numbers.

Mycelial characters of the cultures were noticed and recorded. These were identical to the characters explained by Ahlawat and Tewari (2007). V2, V3, V4 and V13 showed thin and sparse type of mycelial development while other cultures showed thick and fluffy mycelial growth. Evaluation of cultural characters showed that the local isolate collected from Poonkulam (V6) showed faster mycelial growth and it could be considered as the superior isolate (Table 7).

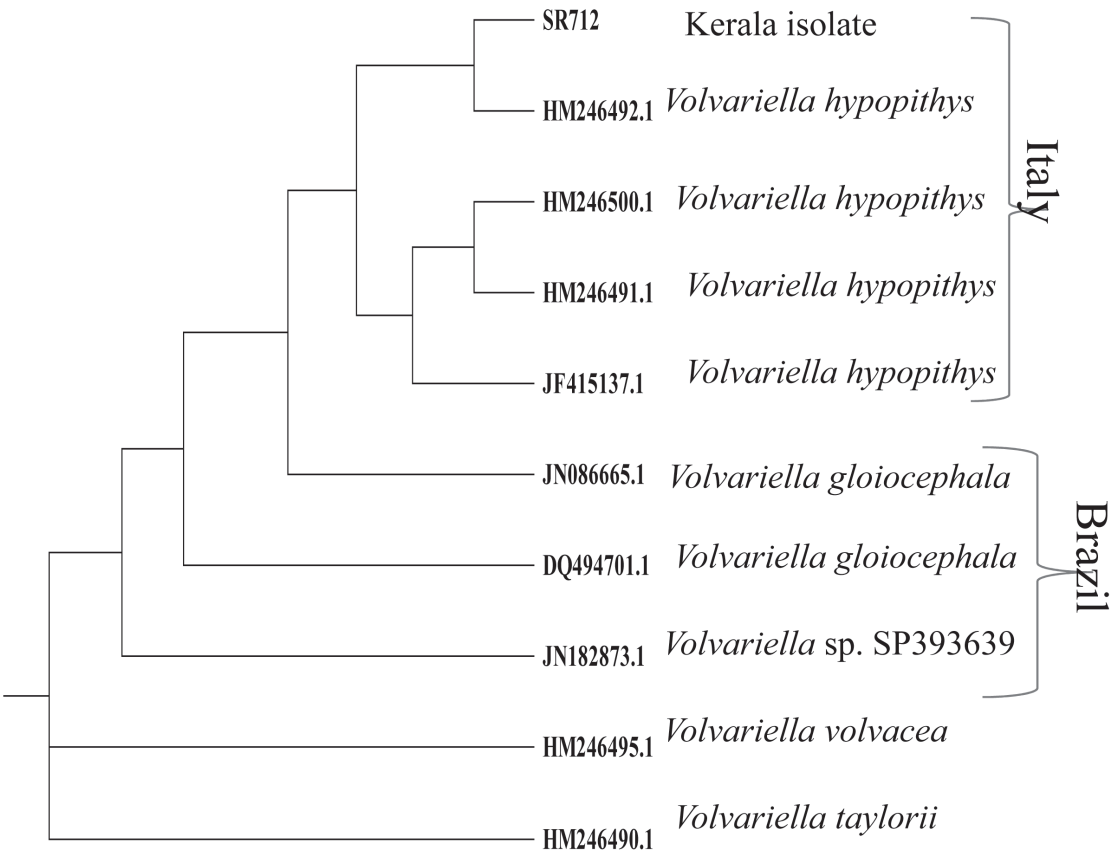

Plate 1. The collected mushrooms in their natural habitat

From the study, a detailed morphological and microscopical understanding of local *Volvariella* collections was made possible. Favourable characters for the cultivation aspects like gregarious occurance, more sporocarp weight, greater cap diameter, faster growth rate, etc. were compared among all the isolates. As an inference the local isolate V6 exceeded in all these favourable production aspects, indicating that it is a superior strain suitable for cultivation in Kerala. The isolate

can be successfully used for spawn production and cultivation during a moderate temperature ranging from 28 - 35° C and at a higher humidity of 85 – 90 %.

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