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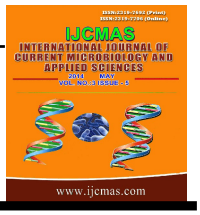

Original Research Article

Molecular characterization of *Volvariella volvacea* (Bull.ex Fr.) Sing strain VVC and analysis of variability among Gen Bank repositories based on ITS region of rDNA

Radhajeyalakshmi Raju*, V.J.Vasanthi, S.Mathiyazhagan, R.Nagarajan, G.Amutha, and R.Velazhahan

Department of Plant Pathology, Centre for Plant Protection Studies,
Tamil Nadu Agricultural University, Coimbatore-641003, India
Corresponding author

A B S T R A C T

Volvariella volvacea (VVC) isolate from Mushroom Research Laboratory, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore was characterized using DNA finger printing techniques. Phylogenetics analysis based on Polymerase Chain Reaction of Internally Transcribed Spacer region and direct sequencing of 5.8S rRNA gene region revealed the interspecific variations of the new isolate with other *Volvariella* isolates deposited by various researchers in Gene Bank National Centre for Biotechnology Information (NCBI database).Multiple sequence alignment exhibited polymorphism in ITS-1 and ITS-2 regions of the new isolate with other accessions but not in the conserved 5.8S rRNA gene regions. The VVC isolate was molecularly characterized as *Volvariella volvacea* and assigned Gen Bank accession number (VVC) by NCBI.The new sequence is now available in public domain.

Keywords

Volvariella volvacea, Internally Transcribed Spacer, PCR, Accession Number, NCBI.

Introduction

Volvariella volvacea, (Bull.exFr.) Sing the Chinese straw mushroom, is an important edible fungus of the tropics and subtropics and is the third most popular cultivated mushroom in the world, which is well known for its pleasant flavour and taste. More than 100 species, sub species and varieties belonging to *Volvariella* have been described from throughout the world. Shaffer, 1957 described 23 species of *Volvariella* based on gross morphology, pink spore, free lamellae and stipe with no annulus at the base by the volva (Singh *et al*. 2003). In case of higher basidiomycetes, morphology and microscopic characters of the basidiocarp are still on the basis of their identification and classification. Nevertheless, highly polymorphic basidiocarp morphology and quite homogenous microscopic characters are insufficient to reveal intraspecific variations. Moreover it is not feasible to

conduct large scale grow-out tests for identification of all the accessions, as it is time consuming procedure. The analysis of genomic DNA using PCR-based methods has proven to be a fast, sensitive and reliable method for determining genetic relationships among basidiomycetes (Singh *et a*l. 2003).

Nuclear rDNA, and particularly the internal transcribed spacer (ITS) regions are good targets for the phylogenetics analysis in fungi (Bruns *et a*l. 1991) because the ITS regions are often highly variable between isolates of the same species (O'Donnell *et a*l. 1988, Salazar *et a*l. 1999).

This work was carried out to investigate the use of ITS-PCR to study the molecular variability among the accessions of *V. volvacea* and VVC of Mushroom Research Laboratory, TNAU, Coimbatore, Tamil Nadu, India.

Materials and Methods

Fungal isolate

An isolate of *V. volvacea* from Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore, India was used in the present study. The mushroom mycelium was maintained in ill filled paddy grains with 2% Horse gram and grown in paddy straw substrate as cylindrical bed system.

DNA extraction

Young Sporocarps (8 days old) were collected from the beds and transferred into an Eppendorf tube containing 500 ml of extraction buffer (50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 0.7 M NaCl; 1% Cetyltrimethylammonium bromide (w/v); and 1% 2-mercaptoethanol) (McDermott *et a*l. 1994) and vortexed for 30 sec and incubated at 60 .C for 1 h. The mixture was centrifuged at 13,000 g for 10 min, and the aqueous phase was added with an equal volume of chloroform: isoamylalcohol (24:1 v/v) and incubated on a shaker (100 rpm) at room temperature (27±2 .C) for 1 h.

The mixture was centrifuged at 13,000 g for 10 min and the aqueous phase was transferred to a new Eppendorf tube and re-extracted with chloroform: isoamylalcohol. The aqueous phase was transferred to a new tube and the DNA was precipitated with an equal volume of isopropanol and centrifuged at 13,000 g for 10 min. The pellet was washed with 70% ethanol, dried and dissolved in 50 ml of Tris-EDTA buffer (10 mM Tris-HCl and 1mM EDTA, pH 8.0).

PCR amplification

The ITS region of VV1 isolate was amplified with primers ITS1, (50-TCC GTA GGT GAA CCT GCG G-30) and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') of White *et a*l. (1990). PCR was under taken in 20 ml volume consisting of 5 mM each dNTPs, 20 pmol each of ITS 1 and ITS 4 primers, 0.5 U of *Taq* DNA polymerase and 100 ng of template DNA. Amplifications were done with the following cycling parameters; 948C for 5 min, 35 cycles of 948C for 2 min, 538C for 2 min, 72 .C for 2 min, and final extension of 30 min at 72°C.

Sequencing and analysis of ITS region

The PCR product of ITS amplified region containing ITS-1, 5.8S rRNA was directly sequenced using ITS-1 (Forward primer) and ITS-4 (Reverse primer). Nucleotide

sequence comparisons were performed by using Basic Local Alignment Search Tool (BLAST) network services against the National Center for Biotechnology Information (NCBI) database. For cluster analysis NTSYS.PC (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) computer program was used. The generated pair wise similarity matrix was used to group strains by the unweighted pair group method arithmetic average (UPGMA). A dendrogram was derived from the similarity matrix. Multiple 5.8S rRNA gene alignment was performed using Clustal x 1.83 software.

Results and Discussion

Genomic DNA was isolated from *V.volvacea* (VVC) and the ITS region, which includes the internal transcribed spacer regions 1 and 2, the 5.8S rRNA genes was amplified with primers ITS1 and ITS 4. PCR amplification of *V. volvacea* (VVC) ITS regions gave products of approximately 700 base pairs in length (Fig.1). The direct DNA sequencing of the PCR product confirmed as *Volvariella volvacea* and compared with other accessions of *V. volvacea* deposited in NCBI by various researchers in the world.

Analysis of the genetic coefficient matrix derived from the scores of ITS profiles showed that minimum and maximum percent similarities among the tested *V. volvacea* strains were in the range of 22 and 94% respectively (Table. 1). Phylogenetics analysis showed that all the isolates compared in this study could be grouped into three main clusters (A, B and C) (Figure 2 and 3). Cluster C consisted of VVC strain and AJ24454 strain of *V. volvacea* having similarity index of 90% between them at molecular level. All the remaining strains belonged to cluster A which consisted of 2 main groups (AY63605 and AY6360**4)** and B consisted of U1597**3.** Chiu and Moore, 1999 recorded similar patterns of PCR of three *V. volvacea* strains. Fungal rDNA has been found to contain regions of variability within genera (White *et al.* 1990, Seifert *et al.* 1995, Levy *et al.* 2001).

Fig. 1.

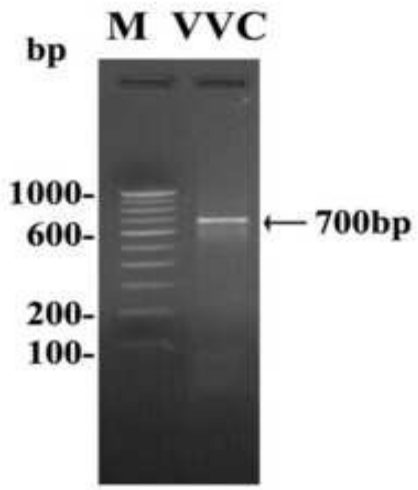

Fig.1. Agarose gel electrophoresis showing internal transcribed spacer 1 (ITS1), 5.8S, and ITS2 ribosomal DNA PCR products of *Volvariella volvacea* (VVC).

The ITS region of nuclear ribosomal DNA, including ITS1, ITS2 and the intervening 5.8S rRNA gene, has been used to determine variability in fungi at the species and subspecies levels (Peterson 1991, Cooke *et al.* 1996, Crawford *et al.* 1996, Cooke and Duncan 1997, Goodwin and Zismann 2001, Appiah *et al.* 2004). Analysis of the PCR-amplified ITS regions of *V.volvacea* (VVC) in the present study confirmed the heterogeneity among the isolates of paddy straw mushroom.

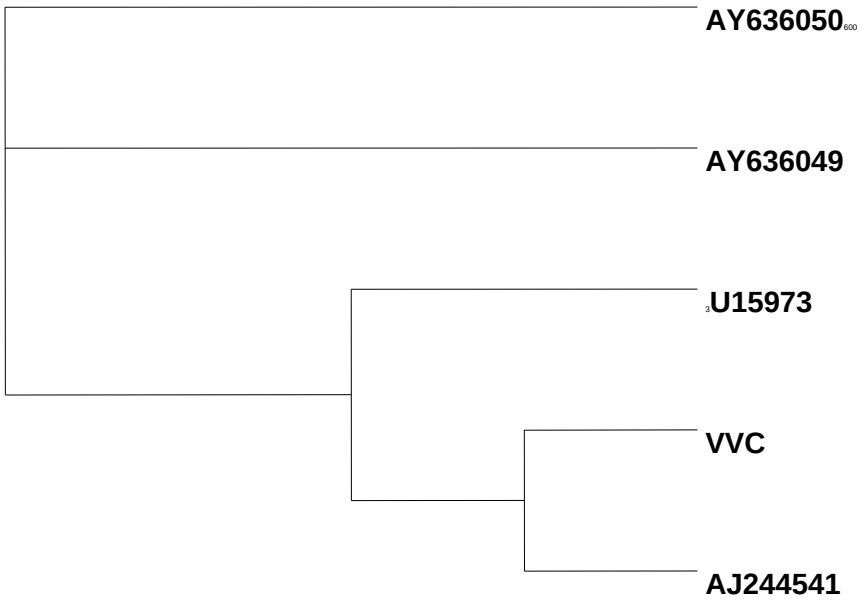

Fig.2. Multiple sequence alignment comparisons of *V.volvacea* (VVC) isolate with other Gen Bank Repositories

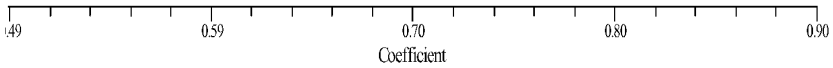

Fig.3 Unweighted pair group method arithmetic average dendrogram constructed from ITS sequence data indicating the relationship among the isolates of *Volvariella volvace*a (VVC).

Table.1 Genetic similarity coefficient matrix for isolates of *Volvariella volvacea* (VVC) based on the ITS regions of the ribosomal DNA

Isolates	**AY636050**	**AY636049**	**U15973**	**VVC**	**AJ244541**
AY636050	1	0.946	0.809	0.465	0.27
AY636049	----	1	0.78	0.476	0.283
U15973	----	----	1	0.427	0.231
VVC	----	----	----	1	0.223
AJ244541	----	----	----	----	1

The genetic variability among the isolates of *V. volvacea* could lead to the occurrence of new strains that could be more nutritive and fast growing. Similar DNA fingerprints of cultivated *V.volvacea* strains were attributes to the genetically homogeneous nature of the fungus (Clemencon, 2004; Moore *et a*l. 2005). The results indicated that PCR products of ITS regions could be used effectively to distinguish the strains of *V. volvacea* and will pave way for handling intellectual property rights.

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