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Morphological and molecular characterization of some popular cultivated mushrooms of India

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ABSTRACT

The mushroom cultivation is a highly lucrative agribusiness to produce nutritious food and earn money in short space and time. The present studies were conducted to analyze morphological and molecular characteristics of five cultivated strains of mushrooms *Agaricus bisporus* (NBS-5), *Calocybe indica* (DMRO-302), *Flammulina velutipes* (DMRO-253), *Pleurotus florida* (DMRP-136) and *Volvariella volvacea* (DMRO-484). The fruiting bodies were procured from ICAR-Directorate of Mushroom Research, Solan, HP (India) and screened for morphological and molecular details. Macroscopic features like shape, size and colour of stipe and pileus were recorded. Microscopic details were worked out by cutting free hand sections of material and observed under research microscope and scanning electron microscope. Molecular identification was done through internal transcribed spacer (ITS) regions of ribosomal DNA sequencing. The nucleotide sequences of each sample were analyzed for Basic local Alignment Search Tool (BLAST) search program in US National Centre for Biotechnology Information (NCBI) for identification. The DNA sequences obtained in this study were submitted in the Gen Bank database at NCBI with accession numbers MW718994.1 (*A. bisporus*), MW714627.1 (*C. indica*), MW719474.1 (*F. velutipes*), MW719254.1 (*P. florida*) and MW720603.1 (*V. volvacea*).

Keywords: Mushroom, molecular identification, internal transcribed spacer, nucleotide sequencing, Gen Bank database

The word mushroom is thought to have been derived from the French word "mousseron" and comprised of agaricoid fungi under order Agaricales of class Agaricomycetes (Kirk *et al*., 2008). Mushrooms are nutritionally rich with high quantity of quality proteins, minerals, trace elements, vitamins and dietary fibers while low in calories and cholesterol (Wani *et al.,* 2010; Rajeshbabu *et al*., 2012; Meng *et al*., 2016). Mushrooms are also known to have many medicinal properties such as antifungal, antibacterial, antiviral, antidiabetic, anticancer, immunomodulation, hepatoprotective, hypolipedimic and hyposensitive activities (Yang and Jong, 1989; Wasser and Weis, 1999; Ooi and Liu, 1999; Wasser, 2002; Jonathan and Fasidi, 2003 and Okhuoya *et al*., 2010).

The mushroom species commonly cultivated throughout the world comprised of white button mushroom (*Agaricus bisporus*) with 11% production share, oyster mushroom (*Pleurotus* spp.) with 21% share, paddy straw mushroom (*Volvariella volvacea*) with 1% share, black ear mushroom (*Auricularia* spp.) with 21%, shiitake (*Lentinula edodes*) with 26%, enoki mushroom (*Flammulina velutipes*) with

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7% share and 13% share for other mushrooms in 2018 (Singh *et al*., 2020). In India, mainly five mushroom species are cultivated with percent production share of 73% white button mushroom (*A. bisporus*), 16% oyster (*Pleurotus* spp.), 7% paddy straw mushroom (*V. volvacea*), 3% milky (*C. indica*) and 1% other mushrooms including shiitake (*L. edodes*) (Sharma *et al*., 2017).

Mushrooms are still identified and classified on the basis of morphological and microscopic characters of the basidiocarp following the description given by Singer (1986) but, due to highly polymorphic basidiocarp and quite homogenous microscopic characters, it becomes difficult to distinguish between similar species without intervention of a professional taxonomist. For identification, diversity analysis and determining genetic relationships among mushrooms, the analysis of genomic DNA using PCR-based methods has proven to be fast, sensitive and reliable (Singh *et al*., 2003). Nuclear ribosomal DNA and highly variable internal transcribed spacer (ITS) regions are good targets for the identification and phylogenetic analysis in fungi (O Donnell *et al*., 1998; Bruns *et* al., 1991; Salazar *et al*., 1999).

Recently molecular techniques along with bioinformatic tools used for fungal identification, diversity analysis and genetic relatedness amongst fungal species include PCR coupled with RFLP (Miller *et al.*, 1999), isozyme analysis (Gottlieb *et al.*, 1998), direct sequencing of the rRNA genes (Moncalvo *et al.*, 1995;Bruns *et al.,* 1991). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA) has been assumed as a universal DNA barcode marker for identification of fungi (Schoch *et al*., 2012). Molecular genetic markers have been employed for rapid identification of different kinds of mushrooms by several workers (Singh, *et al.,* 2003; Lee *et al*., 2006; Yadav *et al.*, 2007; Shukla and Jaitly, 2011 Pushpa *et al.,* 2012; Lallawmsanga *et al.,* 2016; Sagar and Thakur, 2018). The present investigation was undertaken in order to correlate the taxonomic and molecular data for easy and better identification of commonly cultivated mushroom species in India i.e. *Agaricus bisporus* (NBS-5), *Calocybe indica* (DMRO-302), *Flammulina velutipes* (DMRO-253), *Pleurotus florida* (DMRP-136) and *Volvariella volvacea* (DMRO-484).

MATERIALS AND METHODS

Collection of materials

The fruiting bodies of popular strains of edible mushrooms *A. bisporus* (NBS-5), *C. indica* (DMRO-302), *F. velutipes* (DMRO-253), *P. florida* (DMRP-136) and *V. volvacea* (DMRO-484) were obtained in 2020-21 from ICAR- Directorate of Mushroom Research, Solan, Himachal Pradesh, India. Standard cultivation technologies developed by ICAR-DMR Solan (Sharma, *et al*., 2020) were followed to carry out the entire work.

Taxonomic examination

The basidiocarps were studied in detail for various macroscopic features like shape, size, colour, type of gills and margin and microscopic examination like hyphal system, basidia and basidiospores (Singer 1986; Atri and Kaur, 2003 and Upadhyay and Kaur, 2004). Colour descriptions were based on Methuen Handbook of Colours (Kornerup and Wanscher 1978).

Anatomical details related to hyphae, basidia and basidiospores of the specimens and their arrangements were studied by making crush mounts and hand cut sections, staining them with Congo red, cotton blue and Melzer's reagent. Photomicrographs of slides of gills, mycelium and spores were taken and measured with Olympus BX53 microscope. Electron micrographs of gill surface and spores were prepared with Emcrafts Cube 1000/1100 (Manual Stage) table top Scanning electron microscope (SEM).

Isolation of pure culture

The pure cultures were raised from tissue taken from healthy fresh mushrooms. The specimen was first surface sterilized with alcohol and then a small portion of tissue from the mushroom was taken with the help of a sterilized blade and transferred aseptically into the Petri plates containing malt extract medium or potato dextrose agar medium (PDA) and incubated at 25°C for at least 8-10 days and observed regularly. The actively growing mycelial colonies were observed, selected and sub cultured to obtain pure cultures.

Molecular characterization

Molecular characterization of various mushrooms was done by nuclear ribosomal DNA analysis encompassing Internal transcribed spacer region of 5.8S rDNA. The DNA isolation and amplification was done following methods of and White *et al*. (1990).

DNA isolation and visualization

Genomic DNA was isolated from mycelial culture using CTAB method (Doyle and Doyle, 1987). One gram dry lyophilized mushroom mycelium along with pinch of PVP (poly-vinyl pyrrolidone) and fine sand grounded to a fine powder. Added 10 ml pre warmed (65°C) CTAB lysis buffer to it and incubated at 65°C for one hour in water bath with occasional shaking. Extraction was done with Cholorform: Isoamyl alcohol (24:1) twice followed by centrifugation at 5000 rpm for 10 minutes at room temperature (24°C). Top aqueous layer was aspirated in fresh tube, precipitated with 0.6 volume of pre chilled isopropanol and 0.1 volume of sodium acetate (3M), and placed in ice for 10 minutes. The precipitated DNA was palleted with centrifugation at 8000 rpm for 10 minutes at 4°C. DNA pallet was washed with 75% ethanol twice and suspended in 1 ml of 1x TE buffer for overnight. On the next day 2µl of RNAase (10 mg/ml) and Proteinase K (10 mg/ml) were added and incubated for one hour at 37°C. DNA pallet was re-precipitated with 1x volume of sodium acetate (3M) pH 6.8 and 2x volume of pre-chilled absolute ethanol. The purified DNA was re-suspended in 500µl of 1x TE and stored at -20°C till further use.

The isolated DNA was visualized on 0.8% agarose gel and stained with ethidium bromide. The DNA was electrophoresed at 60 volts for 90 minutes and visualized under UV transilluminator.

ITS1-5.8S-ITS2 Amplification

ITS1-5.8S-ITS2 region of rDNA was amplified by polymerase chain reaction (PCR) using universal primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990). The reaction mixture (50µl) contained 10x PCR buffer, 25 mM $MgCl_2$, glycerol 3%, 100 mM of each dNTPs, primers ITS-1, ITS-4, 5 units of Taq DNA polymerase, water and 50 ng DNA. Amplifications were performed in a thermal cycler (Eppendorf) with an initial denaturation step of 95ºC for 2 minutes followed by 34 cycles of 95ºC for 1 minute, 50ºC for 0.45 seconds, 72ºC for 1.20 minute and a final extension of 72ºC for 10 minutes. Control containing no DNA template was included for the presence of contamination of reagents and reaction buffer. Aliquots (5µl) of amplification products were electrophoresed on a 1.6% agarose gel containing ethidium bromide and visualized on a UV transilluminator.

DNA quantification and purification

The quantification of the PCR product was done with Nanodrop lite spectrophotometer (Thermo scientific) and purification was done with PCR purifying kit (Diffinity Rapid Tip®2, Sigma Aldrich).

Sequencing

The amplified DNA fragments were sequenced at Eurofins Genomics India Ltd (Bangalore). Obtained sequences of all the isolates were compared with those available in GenBank databases using BLAST program. The sequences were aligned to

minimize the number of inferred gaps. The sequences were edited with BioEdit 5.0.6 (Hall, 1999) and phylogenetic tree was generated through NCBI website using fast minimum evolution at 75% similarity index. All sequences were submitted to NCBI database.

RESULTS AND DISCUSSION

A. Taxonomical studies

***Agaricus bisporus* (J.E. Lange) Imbach (NBS-5) [Plate-1]**

Plate 1. *A. bisporus* A - C: Fruiting bodies; D: Spore print; E: Basidiospores and F: Basidiospore (Scanning electron microscope SEM); G: T.S. of Gill; H: Hyphae; I Pure culture.

Macroscopic description

Carpophores up to 10 cm high. Pileus 4- 9 cm in diameter, convex to nearly round at first then finally flat, whitish, covered with tiny appressed fibrils or scales, non bruising and pleasant. Lamellae free, initially pinkish then brown, with short frequent lamellulae, 0.3-0.7 cm broad. Stipe 4- 8 cm long, 2-3 cm broad, white with a thin annulus ring, that sometimes disappears in maturity. Spore print dark brown.

Microscopic description

Basidiospores 5.5 8.5 × 4.5 6 µm, ellipsoid, smooth, thick walled and dark brown. Basidia 19 22 × 5 8 µm, clavate, bisporic, sterigmata was up to 5 µm long. Pleurocystidia absent. Cheilocystidia 19 30 × 7 10 µm, clavate and few. Clamp connections absent. Spore print brown.

Remarks

In the present workout sample all the macroscopic and microscopic details show conformity with details given by Wasser SP (2000), Karwa and Rai (2010) and Siddiqui, (2020) for *A. bisporus*. This species is easily identified by its dome shaped to convex shaped cap with free brownish lamellae and annulate stipe.

***Calocybe indica* Purkay. & A. Chandra (DMRO-302) [Plate-2]**

Macroscopic description

Carpophores up to 12 cm high. Pileus up to 13 cm wide, initially convex then flattened at maturity with small umbo, surface whitish with inrolled margin. Lamellae were whitish, unequal, 3 4 lamellulae present, crowded, slightly decurrent. Stipe central, up to 10 cm long, cylindrical with bulbous base measuring up to 3.5 cm in width, with granular appearance over the entire surface. Spore print white.

Microscopic description

Basidiospores were 6-8 × 4 5.5 µm, inamyloid, oval, cyanophilous. Basidia 11-20 × 3-5 µm, clavate, tetrasporic, siderophilous granules present. Cystidia were absent. Pileus surface was with radially arranged cuticle hyphae measuring up to 5.0 µm in width. Clamp connections present.

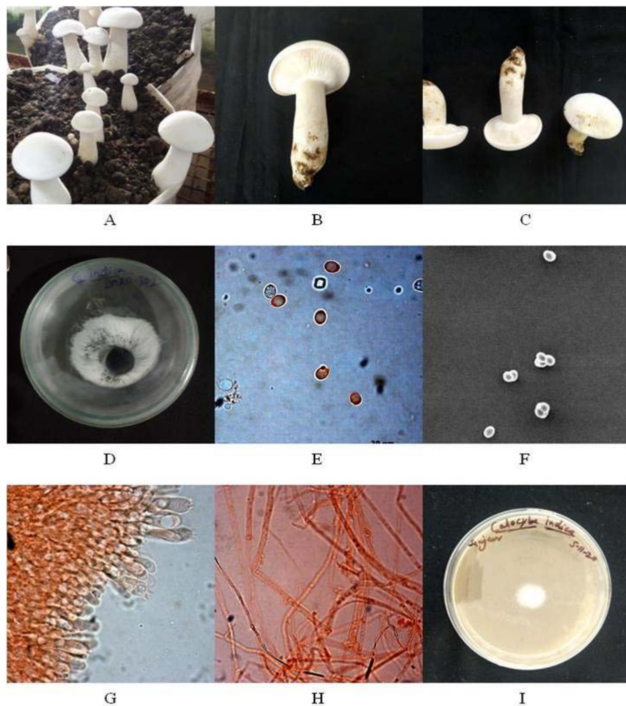

Plate 2. *C. indica* A - C: Fruiting bodies; D: Spore print; E: Basidiospores and F: Basidiospore (SEM); G: T.S. of Gill; H: Hyphae; I Pure culture.

Remarks

Microscopic and macroscopic details show highly remembrance with details given by Purkayastha and Chandra (1974), Bedi *et al*. (2017). It is easily recognized by its campanulate to convex shaped silky white cap with shortly decurrent lamellae and whitish stipe.

Flammulina velutipes **(Curtis) Singer (DMRO-253) [Plate-3]**

Macroscopic description

Carpophores up to 9 cm high. Pileus 3-7 cm wide, broadly convex to finally flat, moist, sticky, dark orange brown to yellowish brown. Lamellae adnate, whitish to yellowish brown, 0.3 cm broad, crowded. Stipe up to 8 cm long, 0.6 cm broad, equal, tough, orange brown to rusty brown, velvety, stuffed. Spore print white.

Macroscopic description

Basidiospores 6 10 × 3.5 5 µm, ellipsoid, inamyloid, smooth. Basidia 24 30 × 10 13 µm, narrowly clavate, thin walled, tetrasporic, sterigmata up to 3 µm long. Pleurocystidia and cheilocystidia both similar in size and shape, scattered, lageniform to ventricose, 28 50 × 10 15 µm, thick walled. Pileipellis a cutis of ixotrichoderm made up of branched, cylindrical to fusoid ventricose elements with clamps up to 15 µm broad. Clamp connections were present.

Remarks

All the microscopic and macroscopic details of *Flammulina velutipes* goes well with description given by Sharma *et al*., (2008) and Davis et al. (2012). It can be easily identified by its orange brown coloured fruiting bodies with long tough stipe often growing on woody material.

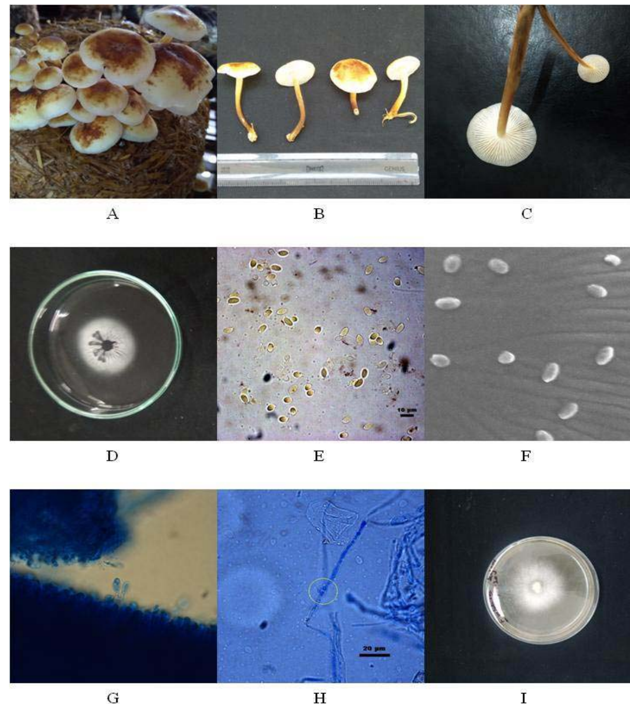

Plate 3. *F. velutipes* A - C: Fruiting bodies; D: Spore print; E: Basidiospores and F: Basidiospore (SEM); G: T.S. of Gill; H: Hyphae; I Pure culture.

Pleurotus florida (Mont.) Singer (DMRP-136) [Plate-4]

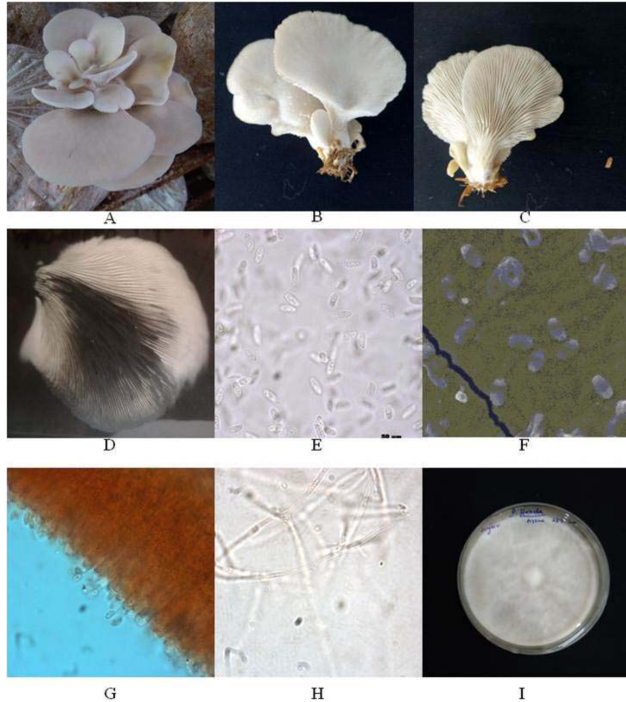

Plate 4. *P. florida* A - C: Fruiting bodies; D: Spore print; E: Basidiospores and F: Basidiospore (SEM); G: T.S. of Gill; H: Hyphae; I Pure culture.

Macroscopic description

Carpophores up to 11 cm high. Pileus 4 -12 cm broad, fan shaped, flabelliform to kidney shaped in out line, white to yellowish white, margin inrolled in young stage. Lamellae were shortly decurrent, running down the stipe, white with greyish tinge. Stipe lateral, up to 3.5 cm long, 2 cm broad, lateral or rudimentary, hairy or velvety. Spore print white.

Microscopic description

Basidiospores were 7-11 × 2-4 µm, cylindric, inamyloid, and smooth. Basidia were 28-45 × 6-9 µm, clavate, tetrasporic; sterigmata up to 6 µm long. Cystidia was absent. Pileipellis a partial ixocutis of tangled cylindrical elements up to 10 µm wide. Clamp connections were present.

Remarks

Microscopic and macroscopic descriptions show full similarity with details given by Davis *et al*. (2012), Shukla, and Jaitly, (2011) and Priyadarshini, (2018). It is easily identified by its fan shaped fruiting bodies with decurrent lamellae and stout stipe. Guzman *et al*. (1994) had suggested that the strains of *P. florida* were the wild isolates of *P. ostreatus*. It was confirmed by phylogenetic analysis based upon the V4, V6 and V9 sequences that *P. ostreatus* and *P. florida* represent a single species (Gonzalez and Labarere, 2000).

Volvariella volvacea (Bull.) Singer (DMRO-484) [Plate-5]

Macroscopic description

Carpophores up to 15 cm high. Pileus 9-15 cm broad, initially egg shaped, convex, bell-shaped to nearly flat, whitish to greyish, covered with brown

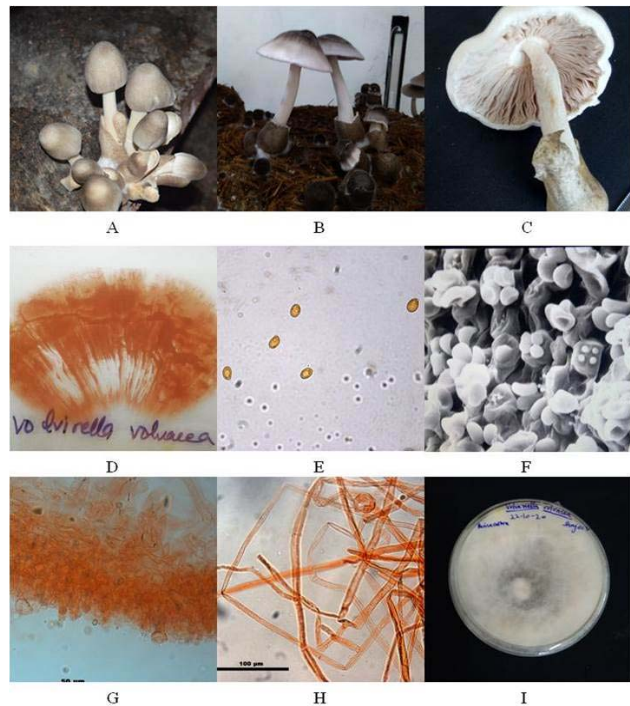

Plate 5. *V. volvacea* A - C: Fruiting bodies; D: Spore print; E: Basidiospores and F: Basidiospore (SEM); G: T.S. of Gill; H: Hyphae; I Pure culture.

silky hairs, fibrillose. Lamellae free, crowded, with short frequent lamellulae, brownish pink and 0.6-0.8 cm broad. Stipe central, 9-14 cm long, 1-2 cm thick, equal, sometimes tapering upwards, whitish, basal saccate yellowish brown volva present. Spore print salmon pink.

Microscopic description

Basidiospores 6-9 × 3.5-6 µm, ellipsoid, inamyloid. Basidia 18-40 × 6-10 µm, clavate, tetrasporic, sterigmata up to 4 µm long. Pleurocystidia and cheilocystida 30-70 × 15-25 µm, variable in shapes, abundant, subglobose, pyriform, to lageniform. Clamp connections absent.

Remarks

It can be easily identified by its medium sized fruiting bodies with convex shaped to campanulate grayish brown cap with pinkish brown lamellae and volvate stipe. Microscopic and macroscopic descriptions show full similarity with details given by Breitenbach & Kranzlin (1991), Bedi *et al*., (2017) and Ali *et al*., (2017).

B. Cultural Characteristics

Pure cultures of different strains of cultivated mushrooms were raised from stipe and pileus of healthy fresh fruiting bodies on malt extract and PDA medium. Pure culture was obtained after 7-10 days incubation at 25±2°C in *A. bisporus, F. velutipes, P. florida* and *V. volvacea*. Pure culture of *C. indica* was obtained after 14 days incubation at 30±2°C. Mycelial growth was radial, white, becomes cotton matted in all mushrooms except *V. volvacea* which have dense aerial hyphae of creamy white colour.

C. Molecular Characterization

Amplified products of ITS-5.8s rDNA is shown in Fig. 1. The concentration of DNA in PCR product was 119.8 ng/µl in *A. bisporus*, 136.9 ng/ µl *C. indica,* 251.6 ng/ µl in *F. velutipes,* 141.8 ng/ µl in *P. florida and* 163.8 ng/ µl in *V. volvacea.* Curation of the sequence data showed sequences of ITS-1, 5.8S, ITS-2 and partial sequences of small and large subunit ribosomal RNA genes. Identity of five cultivated mushroom strains was confirmed by performing Basic Local Alignment Search Tool (BLAST) analysis against NCBI database. The PCR-based methods have proven to be a fast, sensitive and reliable method for identification, classification and phylogeny of mushrooms (Singh *et al*., 2003). Lallawmsanga *et al.* (2016) characterized forty five isolates of wild mushrooms through ITS rRNA gene amplification and classified the isolates into 16 genera belonging to 11 families.

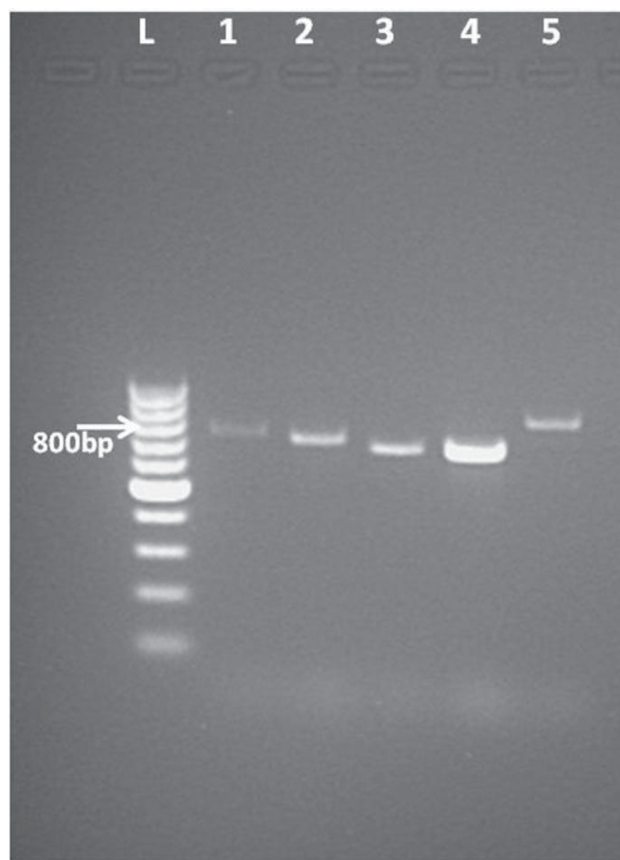

Fig. 1. 100bp ladder; 1: *A. bisporus;* 2: *V. volvacea*; 3: *P. florida*; 4: *C. indica*; 5: *F. velutipes*

A. bisporus (NBS-5)

PCR amplified product of ITS 5.8S rDNA of *A. bisporus* (NBS-5) was approximately 703 base pairs

in length. The direct DNA sequencing of the PCR product confirmed as *A. bisporus* through BLAST and compared with other accessions of *A. bisporus* deposited in NCBI by various researchers in the world. ITS sequence isolated from *A. bisporus* (NBS-5) showed 99.56% similarity with accession number CP015465.1 (*A. bisporus*) an isolate from Netherland (Sonnenberg *et al*., 2016) and accession number JN234842.1 (*A. bisporus*) an isolate from Malaysia (Avin *et al*., 2013). The DNA sequences obtained in this study were submitted through accession number MW718994.1 in the GenBank database. The phylogenetic tree (Fig. 2) was generated through NCBI website using fast minimum evolution at 75% similarity index. The analysis showed most close relationship with *A. bisporus* but at the same time the isolate made an out group from other strains of the fungus. The query coverage is 98% with 99.57 percent identity with accession number CP015465.1 (*A. bisporus*) and JN234842.1 (*A. bisporus*) and the e-value was 0.00, which showed that the isolate is *A. bisporus* but also has diversity with other isolates. NBS-5 Strain of *A. bisporus* is a browning-resistant hybrid developed by mating two genetically diverse strains and molecularly characterized for identification of markers and confirmation of hybridization (Kamal *et al*., 2019). Molecular tools have been proved to be helpful in differentiating wild and cultivated *Agaricus* species (Mozina *et al*., 1993; Kerrigan, 1995). Junang *et al*. (2009) studied the rDNA ITS sequences of two high quality strains Tb07 and As2796 of of *A. bisporus* and suggested to screen the variations among strains to utilize for cross breeding.

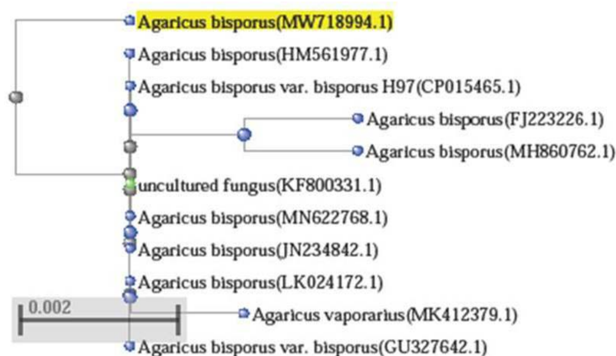

Fig. 2. Genetic relatedness of NBS-5 strain with other isolates of *A. bisporus* using fast minimum evolution method

C. indica (DMRO-302)

The PCR amplified product of ITS 5.8S rDNA of *C. indica* strain DMRO-302 was of approximately 660 base pairs in length. Nucleotide comparison of sequences of the ITS 5.8SrDNA using BLAST against NCBI database with other accessions of *C. indica* in NCBI showed 98.64% similarity with accession number MT636315.1 (*C. indica*), an isolate from South Korea (Min and Lee, 2020) and 98.56% similarity with accession number MF401593.1 (*Macrocybe gigantea*), an isolate from India (Mariselvi and Earanna, 2018). The DNA sequences obtained in this study were submitted through accession number MW714627.1 in the GenBank database. Both *Calocybe* and *Macrocybe* have close resemblance and in India the larger size and white colour of both genera are confusing and lead to misidentification (Razaq *et al*., 2016). Previously, the members of *Macrocybe* were treated in *Tricholoma* sect. *Leucorigida* Singer (1986), but the type specimen of this section belongs to *Calocybe* (Pegler *et al*. 1998). Pegler *et al*. (1998) segregated *Macrocybe* from *Tricholoma* and ranked it to a genus. The phylogenetic tree (Fig. 3) generated through NCBI website using fast minimum evolution at 75% similarity index showed that most close

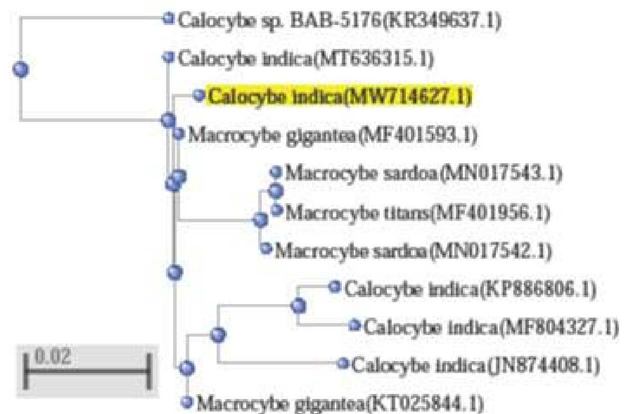

Fig. 3. Genetic relatedness of DMRO-302 strain with other isolates of *C. indica* using fast minimum evolution method

relationship with *C. indica* but at the same time the isolate made an out group from other strains of the fungus. The query coverage was 100% with 98.64 percent identity with MT636315.1 (*C. indica*) and the e-value was 0.00, which showed that the isolate is *C. indica* but also has diversity with other isolates.

F. velutipes (DMRO-253)

The PCR amplification regions of *F. velutipes* (DMRO-253) gave products of approximately 776 base pairs in length. Sequence comparisons confirmed the isolate as *F. velutipes* through BLAST and comparisons with other accessions in NCBI showed 99.61% similarity with accession number KJ999145.1 (*F. velutipes*) an isolate from China (Zhang, 2014), EF595853.1 (*F. velutipes*) an isolate from China (Ge *et al*., 2009), 99.36% similarity with accession number MH469686.1 (*F. velutipes* var. *filiformis*) an isolate from China (Li, 2019) and 98.58% similarity with accession number DQ978220.1 (*F. velutipes*) an isolate from India (Gulati *et al*., 2006). The DNA sequences obtained in this study were submitted through accession number MW719474.1 in the GenBank database. The cultivated strains and wild type strains of *F. velutipes* were distinguished on the basis of RFLP (restriction fragment length polymorphism) analysis of ITS-PCR products (Palapala *et al*., 2002). Ge *et al*., (2008) had suggested on the bases of ITS/5.8S r DNA sequences analysis that the *F. velutipes* from China are more closely related to a Canadian species than European species. The phylogenetic tree (Fig. 4) was generated

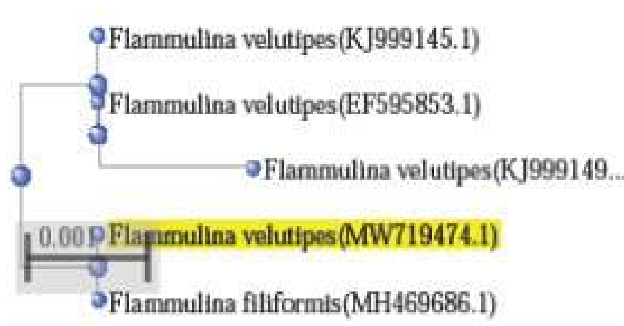

Fig. 4. Genetic relatedness of DMRO-253 strain with other isolates of *F. velutipes* using fast minimum evolution method

through NCBI website using fast minimum evolution at 75% similarity index. The analysis showed that most close relationship with *F. velutipes* var. *filiformis* but at the same time the isolate made an out group from other strains of the fungus. The query coverage was 99% with 99.36 percent identity with MH469686.1 (*F. velutipes* var. *filiformis*) and the e-value is 0.00, which showed that the isolate was *F. velutipes* but also has diversity with other isolates.

P. florida (DMRP-136)

The PCR amplification regions of *P. florida* (DMRP-136) gave products of approximately 682 base pairs in length. BLAST analysis of the sequences confirmed the isolate as *P. florida*. ITS sequence showed 100% similarity with accession number MH287458.1 (*P. floridanus*) an isolate from India (Das *et al*., 2018), accession number LC149608.1 (*P. ostreatus*) an isolate from Nepal (Tamrakar *et al*. 2016) and 99.71% similarity with accession number MG819636.1 (*P. floridanus*) an isolate from India (Barh *et al.,*2018). The DNA sequences obtained in this study were submitted through accession number MW719254.1 in the GenBank database. Ma and Luo (2002) utilized the ITS-RFLP for genotype identification of genus *Pleurotus*. Krishnapriya *et al*. (2017) identified four species of Oyster mushroom (*P. eous, P. florida, P. opuntiae and P. cystidiosus*) from India, based on the ITS and nuclear large subunit r DNA sequences. The phylogenetic analysis of *P. ostreatus* and *P. florida* confirmed that they belong to same species (Guzman *et al*. 1994; Gonzalez and Labarere, 2000). The phylogenetic tree (Fig 5) was generated through NCBI website using fast minimum evolution at 75% similarity index. The analysis showed that most close relationship with *P. ostreatus* and *P. floridanus* but at the same time the isolate made an out group from other strains of the fungus. The query coverage was 99% with 100 percent identity with MH287458.1 (*P. floridanus*) and LC149608.1 (*P. ostreatus*) and the e-value was 0.00, which showed that the isolate is *P. florida* but also has diversity with other isolates.

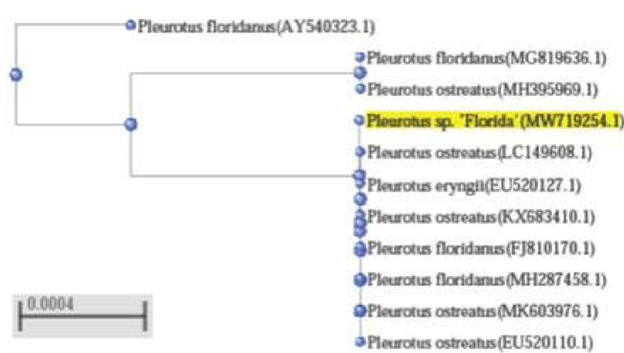

Fig. 5. Genetic relatedness of DMRO-136 strain with other isolates of *P. florida* using fast minimum evolution method

V. volvacea (DMRO-484)

PCR amplification product of *V. volvacea* (DMRO-484) produced approximately 716 base pairs in length. BLAST analysis of the sequence against NCBI database confirmed the isolate as *V. volvacea* ITS sequence isolated from *V.volvacea* (DMRO-484) showed 99.16% similarity with accession number MW077524.1 (*V. volvacea*) an isolate from Viet Nam (Dung*, et al*., 2020) and 99.07% similarity with accession number JN086680.1 (*V. volvacea*) an isolate from India (Ahlawat, *et al.,*2012). The DNA sequences obtained in this study were submitted through accession number MW720603.1 in the GenBank database. Various strains of *V. volvacea* were screened for biological, morphological, and molecular characteristics at Directorate of Mushrooms Research in India (Ahlawat *et al*., 2010; Ahlawat and Savoie 2014; Ahlawat and Kaur, 2018). The phylogenetic tree (Fig. 6) was generated through NCBI website using fast minimum evolution at 75% similarity index. The analysis showed that most close relationship with *V. volvacea* but at the same time the isolate made an out group from other strains of the fungus. The query coverage was 99% with 99.16 percent identity with MW077524.1 (*V. volvacea*) and the e-value was 0.00, which showed that the isolate is *V. volvacea* but also has diversity with other isolates.

Fig. 6. Genetic relatedness of DMRO-484 strain with other isolates of *V. volvacea* using fast minimum evolution method

The present study also reports that GenBank data base for mushroom is not much rich in India. However, molecular characterization of five cultivated mushroom samples up to species level is performed and submitted nucleotide sequences may be relevant to GenBank data base for further exploration in the field.

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