Mysore J. Agric. Sci., 50 (2) : 392-394, 2016

Molecular Identification of the Lentinus Mushroom Documented from the Western Ghats of Karnataka

K. N. Prabhu and N. Earanna
Department of Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru-560065

Abstract

The study was conducted with an objective of documenting and conservating the wild edible mushroom of Western Ghats of Karnataka. Mushroom species were collected during the monsoon season from the Nettana forest, Dakshina Kannada district and field data was recorded. Mushroom DNA was isolated and amplified by using ITS1 and ITS4 primers and sequenced. The sequence alignment was made with sequence available in the National Centre for Biotechnological Information (NCBI). The Blast search analysis indicated 98 per cent homology with the Lentinus sajorcaju Fr.). Pure culture (mycelium) of the mushroom was obtained through tissue culture on potato dextrose agar (PDA) and attempts were made to cultivate the mushroom on paddy straw and sawdust mix in the ratio of 1:1(w/w). Mycelium produced fruiting body at 60 days after spawning. Further, the fruiting body of the mushroom contained 25.55 per cent crude protein.

Western ghats of Karnataka (India) constitute a mountain range along the western coast of India. It is one of the eight hotspots of biological diversity in the world. Humid weather and high rainfall during monsoon is a congenial atmosphere for the establishment of many kinds of mushroom flora which includes edible, medicinal and poisonous types. One third of fungal diversity of the globe exists in India and Only a fraction of this fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the unexplored and hidden wealth. (Manoharachary *et al.,* 2005). There is a need for concerted effort to document the wealth of mushroom biodiversity so that a national database can be developed, which can ultimately help in building up of a global mushroom database of India (Pandey and Veena, 2012). Mushroom contains a substantial amount of dietary fibers, proteins, vitamins and minerals, a good nutritional compound in dietary system (Shirur and Shivalingegowda, 2015).

Mushroom was collected during the rainy season (August 2015) and field information of the mushroom such as habitat, abounndance, size, shape, color of the fruiting body, arrangement of the gills were recorded (Arora, 1986). DNA was extracted from the mushroom tissue by using CTAB lysis buffer. The tissue (100 mg) was ground in to fine powder using liquid nitrogen and the sample was transferred into 1.5ml of extraction buffer and incubated at 65°C for 45 minutes. Equal volumes of chloroform: Isoamylalchol (24:1 v/v) was added and mixed by inverting by tubes. These tubes were centrifuged at 10000 rpm for 10 minutes. Clear supernatant was collected and DNA was precipitated by adding chilled isopropanol. The pellet was washed with 70 per cent ethanol and dissolved in TE buffer. The concentration and purity were measured by using 280 nm and 260 nm / 280nm, respectively in biospectrometer (Eppendorf).

Amplification of 5.8S rRNA gene and ITS region was done by using ITS1 (Forward primer) 5'TCCGTAGGTGAACCTGCGG3' and ITS4 (Reverse primer) 5'TCCTCCGCTTATTGATATGC3' (Rajaratnam and Thiagarajan, 2012). Polymerase chain reaction was performed in a 40µl reaction volume containing 1x Taq buffer with $MgCl_2$ (1.5mm), dNTPs (200µM), forward and reverse primers (0.5 µM each), Taq DNA polymerase (3U) and template DNA (50ng). Amplification was carried out with an initial denaturation at 95°C for 4 minutes, followed by denaturation at 94°C for 1minute, annealing at 59°C for 30 seconds and extension at 72°C (these three steps for 35 cycles) and a final extension at 72°C for 10 minutes. Amplicon were separated on agarose gel (1%) and documented using gel documentation unit (Alpha Innotech). Amplicon was isolated and purified

by using Genjet Elution kit™ (Thermo Scientific), sequenced (Scigenom Pvt. Ltd., Kerala) and compared with sequence in National Centre for Biotechnology Information (NCBI).

Spawn production was done on sourghum with 2 per cent of each calcium carbonate and calcium sulphate. Mushroom was grown on 1:1 (w/w) proportion of sawdust and paddy straw. One gram of dried sample was digested in 15ml of H_2SO_4 containing 5 gm of K_2SO_4 and 0.5mg of $CuSO_4$ at 180°C for 15min, 250°C for 30 min, 380°C for 30 min and 450°C for 90 min. The digest was distilled using 40 per cent NaOH and liberated ammonia was trapped in 4 per cent boric acid. It was tritrated against 0.1N H_2SO_4 and then protein content was determined by multiplying the nitrogen value with 6.25 (Raghuramulu *et al.,* 2003).

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Initial tree for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The analysis involved ITS sequence of *L. sajor-caju* and other 15 differrent species of the genus *Lentinus.* Evolutionary analyses were conducted in MEGA6 (Tamura *et al.,* 2013).

The Fig. 1a reveled that the Habitat of the mushroom is on dead wood, the growth habit is scattered; Colour of the mushroom cap is light Brown and stem is white; Cap is depressed at the center, Cap diameter was 7-12 cm, Stipe is equal which measures 4-6cm in length, 2-3mm in thickness; Medium in texture; Stipe Position was central; Gills wer Decurrent towards the stalk, Annulus was absent; spore print,was white in color; Structure of the cap cuticle is filamentous. The Fig. 1b reveled that Spore were rod like structure, which were white in color

The sequence data was showed 98 per cent homology for *Lentinus sajor-caju* Fr. in the NCBI

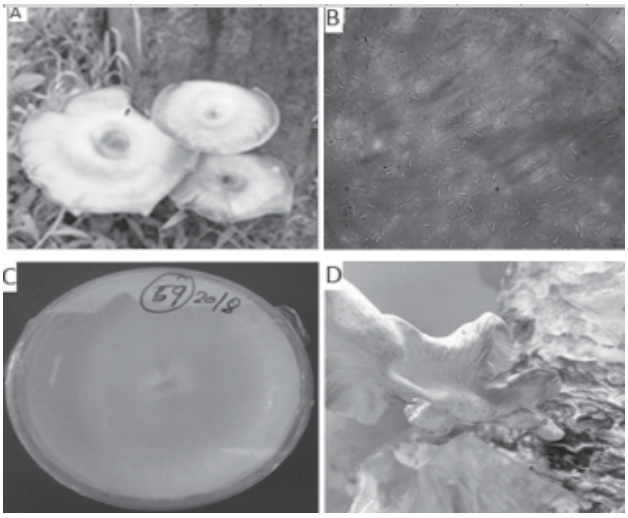

Fig. 1. A) Mushroom grown naturally on the stem of the dead tree; B) Microscopic view of the spores (400 × magnification); C) Pure culture of Lentinus sajor-caju on potato dextrose agar medium; d) cultivation of mushroom on 50% paddy straw and 50% sawdust.

nucleotide blast. Therefore, the mushroom was confirmed as *L. sajor-caju* and it belongs to the family polyporaceae of the order polyporales in the class Basidiomycets. We got the accession number KX181288 for the sequence.

Pure culture of the mushroom was obtained on Potato dextrose agar medium (Fig.1C) which further used for the spawn production. This cultivated

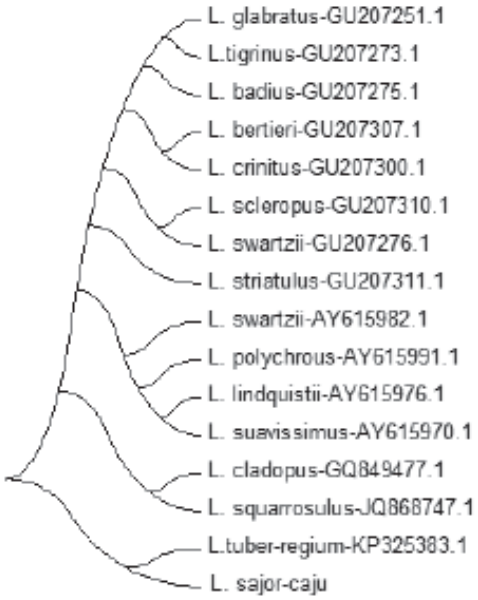

Fig. 2. Molecular Phylogenetic analysis of L. sajor-caju with fifteen different species of the same genera by Maximum Likelihood method.

mushroom produced the fruiting body on 60 days after spawing (Fig. 1d). and contained 25.55 per cent of protein.

The Fig.2 reveled that there were eight branches in *genus Lentinus* in which *Lentinus sajor- caju* (marked with dark triangle in Fig.2) is closely evolved with *Lentinus regium. L. glabratus and L.tigrinus* were distinctly evolved from the *L. sajor-caju.*

References

Aroroa, D., 1986, Mushroom demystified: A comprehensive guide to the fleshy fungi. Ten Speed Press, Crown Publishing Group, New York, pp:8-20.

Felsenstein, J., 1985., Confidence limits on phylogenies: An approach using the bootstrap. Evol., 39:783-791.

Manoharachary, C., Sridhar, K., Singh, R., Adholeya, A., Suryanarayanan, T. S., Rawat, S. and Johri, B. N., 2005, Fungal biodiversity: distribution, conservation and prospecting of fungi from India. *Curr. Sci.,* **89**(1) : 58.

Nei, M. and Kumar S., 2000, Molecular Evolution and Phylogenetics. Oxford University Press, New York, pp :16-23.

Pandey, M. and Veena, S. S., 2012, Characterization and conservation of edible and medicinal mushrooms of Western Ghats of India, Indian J. Trop. Biodiv., 20(1):37-44.

Raghuramulu, N., Nair, K. M. and Kalyanasundaram, S., 2003, Fiber: Food analysis In: A manual of Laboratory techniques, National Institute of Nutrition, Hyderbad, pp : 7-57.

Rajaratnam, S. and Thiagarajan, T., 2012, Molecular characterization of wild mushroom. *Euro. J. Exp. Biol.,* **2**(2) : 369-373.

Shirur, M. and Shivalinge gowda, N. S., 2015, Mushroom marketing channels and consumer behavior: a critical analysis. *Mysore J. Agric. Sci.,* **49**(2) : 390-393.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013, MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol., **30** : 2725-2729.

(Received : May, 2016 **Accepted :** June, 2016**)**