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Morpho-Cultural, Molecular and Nutraceutical Studies on Coremiopleurotus from India

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Abstract: Coremiopleurotus (*Pleurotus cystidiosus* O.K. Miller) is studied for its taxonomic, cultural, molecular features and nutraceutical attributes and illustrated based on collection from Patiala in Punjab (India). It is a wood decaying edible basidiomycetous mushroom, characterized by the formation of specialized anamorphic structures called coremia. Internal transcribed spacer (ITS) region sequencing studies carried out showed highest identity with *Pleurotus cystidiosus* strain ACCC 51280. The aerial mycelium of the mushroom (anamorph) form toxocyst, which is a specialized feature of *P. cystidiosus* subsp. *abalonus* Zervakis, a hitherto unreported variant of dhingri mushroom from India.

Key words: Coremiopleurotus · *Pleurotus cystidiosus* · Taxonomy · ITS sequencing · Nutraceutical studies

INTRODUCTION

Coremiopleurotus belongs the genus *Pleurotus* (Fr.) P. Kumm., which is a member of family Pleurotaceae of the order Agaricales [1].Taxonomically coremiopleurotus is represented by *P. cystidiosus* O.K.Miller, which was earlier documented from India by Natarajan and Raaman [2]. The presently described collection is typical of *P. cystidiosus* subsp. *abalonus* Zervakis, whose anamorphic stage forms toxocyst, which is a specialized feature of this variant. Presently worked out mushroom was found growing in caespitose clusters on the live stump of *Mangifera indica* in the beginning of June, 2009 from Patiala in Punjab which falls within the geographical limits of 30° 20′ North Latitude and 76° 24′ East Longitude in the Indian subcontinent. It has been described based on the morphological features, cultural studies, neutraceutical evaluation and ITS region characterization of the specimen.

MATERIALS AND METHODS

Taxonomic Examination: Methods for collection, preservation and description of agarics have been followed as per standard techniques [3]. Macroscopic examination was carried out on fresh specimens in the field itself. Microscopic characters were studied from free hand sections mounted in 5% KOH, stained with 1% Congo red. Colors in description are based on Methuen Handbook of Colors [4]. The specimens have been deposited in the Herbarium of Botany Department, Punjabi University Patiala (PUN) under accession number 3949.

Culturing: After observing all the morphological characters, the carpophore tissue was taken from the point of junction of stipe with the pileus and sterilized by dipping in 0.1 % mercuric chloride for few seconds. After thoroughly washing in sterilized distilled water the fungal tissue was inoculated on potato dextrose agar medium. The optimum temperature for mycelium growth was recorded at 25 ±1°C. There was no coremia formation below 15°C.

ITS- Sequencing: Five day old mycelium was scrapped from the Petri dishes, frozen in liquid nitrogen and ground to a fine powder. DNA was extracted using Qiagen Plant DNeasy Kit. The amplification of internal transcribed spacer 1, the 5.8 ribosomal RNA gene and internal transcribed spacer 2 was achieved using primers ITS1 and ITS4 [5]. The PCR reaction was performed in 50 µl total volume including 50 ng genomic DNA, 10 pmol of each primer, 0.5 mM of dNTPs, 1 × PCR buffer with 1.5 mM $MgCl_2$ and 3 U Taq polymerase. The thermocycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 35 amplification cycles at 94°C for

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1 min, 54°C for 1 min and 72°C for 2 min and a final extension at 72°C for 8 min. The sequence of the PCR-product was determined by employing the ABI Prism Big Dye Terminator v. 3.1 Cycle Sequencing Kit. The sequence was analyzed using the gapped BLASTn http://www.ncbi.nlm.nih.gov search algorithm and aligned to the nearest neighbours. The evolutionary distances among strain 2001 and its related taxa were calculated with TREECON software package version 1.3b (Copyright © Yves Van de Peer, University of Antwerp, 1994, 1998) using Kimura's two-parameter model, after aligning the sequences with ClustalW. The ITS region sequence of *Agaricus bisporus* voucher GAL 17591 was used as the outgroup. The newly generated sequence was deposited at GenBank (accession no GU947713).

Nutritional and Nutraceutical Evaluation: Proximate composition with respect to proteins, fats, carbohydrates and fibers of the carpophore/100 gm. of the dried sample was determined by using AOAC procedures [6]. Macro and micro-mineral composition was estimated by Jackson method [7] using Atomic absorption spectrophotometer. Besides the mushroom carpophore was also evaluated for the presence of Ascorbic acid, β carotene and Phenolic compounds following standard techniques.

RESULTS

Taxonomic Study: *Pleurotus cystidiosus* O.K. Miller subsp. *abalonus* (Han, Chen and Cheng) G.J. Zervakis Mycol., 90(6): 1063-1074, 1998.

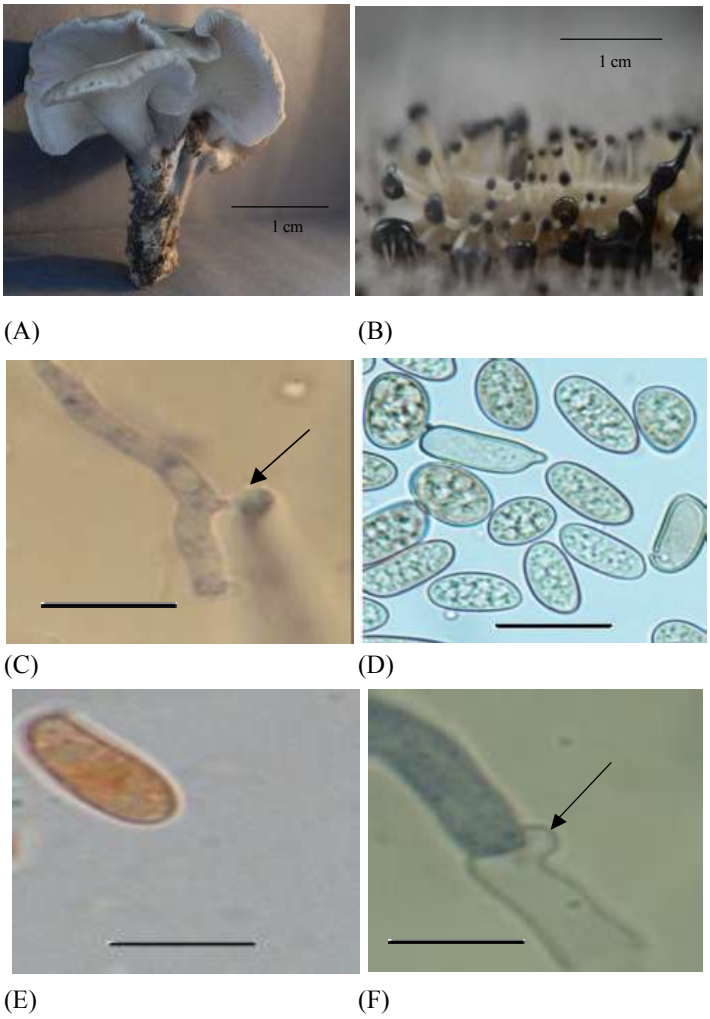

Fig. 1(A-F): (A) Basidiocarp of *Pleurotus cystidiosus* subsp. *abalonus* (B). Coremia formation on the tissue (C) Toxocyst on mycelium (Scale bar=10µm) (D). Coremial spores (Scale bar=10µm) (E) Basidiospore (Scale bar=10µm) (F). Clamp connection on hypha(Scale bar=10µm)

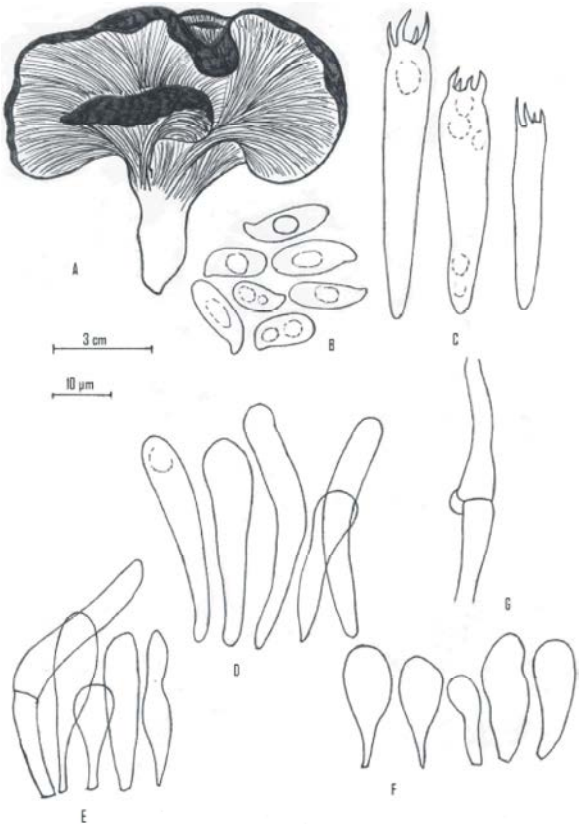

Fig. 2(A-G): A. Carpophore of *Pleurotus cystidiosus* subsp. *abalonus*, *B. Basidiospores, C. Basidia, D. Pleurocystidia, E. Pileocystidia, F. Cheilocystidia, G. Hypha* with clamp connection

Fructification up to 9.2 cm in height, pleurotoid (Figures 1/A & 2/A). Pileus up to 10.5 cm in diameter, depressed; surface grayish brown (8F3) bearing brownish to purplish squamules, dry; veil absent; fleshy, flesh up to 0.3 cm thick, white, unchanging on exposure to air; taste and odour mild. Lamellae yellowish white (4A2), decurrent, extending down on to the stipe, subdistant (up to 0.4 cm apart from each other), unequal, divided into two tiers, ventricose, gill edges wavy, spore print white (1F1). Stipe grayish brown (8F3) lateral, 4.5 to 6.5 cm long, 2.2 to 3 cm broad, tapering downwards, solid, flesh white underneath. Spores 8.5 - 14.5 x 4.5 - 6.64 µm (Q=2.06), oblong, elliptical, inamyloid (Fig. 1/E & 2/B). Basidia 33.2 - 49.8 x 4.98 - 8.30 µm (Figure 2/C). Sterigmata 3.3 - 8.3 µm long; gill edges heteromorphous. Cheilocystidia clavate to pyriform, club shaped, 16 - 22.5 x 5 - 8 µm (Figures 2/F). Pleurocystidia abundant, clavate to ventricose, 36.5 - 43 x 4.98 - 8.3 µm (Figures 2/D). Pileocystidia, abundant, clavate to cylindrical, scattered to aggregated in loose fascicles on the pileus (Figure 2/E). Pileus cuticle hyphal with scattered to aggregated, clavate to cylindrical pielocystidia measuring 24.15 - 32 x 4.83 - 7.3 µm. Clamp connections present in the hyphal elements of pileus (Figure 2/G). Hymenophoral trama composed of interwoven hyphal elements. Stipe hyphae with thick walls.

Culture Characteristics: After 4 days of inoculation of the fresh tissue at 25°C, irregular mycelial growth started on the entire inoculated tissue. Coremia formation started in the form of small protuberances on the entire tissue simultaneously (Figure 1/B). Initially a little white stalk and tiny watery droplets having blackish color appeared on the white stalk. After 3 days of protuberance appearance these terminated into distinct capitate structures each with a white stalk and black head. Microscopical observation of aerial hyphae showed the special globular structures called toxocyst (Figure 1/C), which is a specialized feature of *P. cystidiosus* subsp. *abalonus*. In the hyphae prominent clamp connection was observed (Figure 1/F). Growth of mycelium was cottony and irregular. Coremial spores measured from 7.77 - 10.81 µm (Figure 1/D).

Table 1: Nutrient composition of *Pleurotus cystidiosus* subsp. *abalonus*

Pleurotus cystidiosus (%)	Proteins	Crude fat	Fibers	Ash	Carbohydrates
	3.100±0.4	0.80±0.2	3.12±0.21	2.00±0.7	85.86±0.29

Table 2: Macro and micro mineral composition of *P. cystidiosus* subsp. *abalonus*

Macro-minerals (mg/100g)				Micro-minerals (mg/100g)		
Ca	Mg	Na	K	Cu	Zn	Fe
3.4±0.1	1.284±0.3	1.120±0.1	.0821±0.00	2±0.4	6.21±0.23	20.24±0.12

Table 2: Antioxidants of *P. cystidiosus* subsp. *abalonus*

Ascorbic acid (mg/100g)	Beta carotene (ug/100 gms)	Lycopene (ug/100 gms)	Phenolic compound mg / 100 gms of gallic acid
0.49±0.3	0.22±0.1	0.055±0.2	6.39±0.24

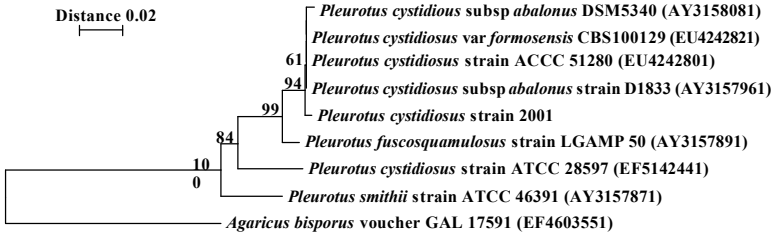

Fig. 3: Phylogenetic tree showing the relationships among *Pleurotus cystidiosus* strain 2001 and representatives of related taxa, based on the ITS region sequences. The numbers on the nodes indicate how often (no. of times, %) the species to the right are grouped together in 100 bootstrap samples. Bar 0.02 substitutions per site

Collection Examined: Baradari Gardens (250m), Patiala, Punjab, India, growing in caespitose clusters on *Mangifera indica*, Sapan Kumar, 3949 accession number under PUN, June 6, 2008, Culture deposition no. MTCC 10940.

Nutritional and Neutraceutical Investigation: Nutritionally this mushroom is rich in all the essential nutrient component including carbohydrates (85.86%), proteins (3.1%), fibres (3.12%) and ash (2%). Besides this, it has low fat (0.8%) contents (Table 1). The results are in agreement with other common edible mushrooms [8]. The low fat value of this mushroom is in consonance with previous reports on edible mushrooms [9]. It is also rich in macro and microminerals (Table 2). The results achieved are in conformity with those reported for other cultivated mushrooms [10].

Antioxidant potential of this mushroom is due to presence of ascorbic acid, carotenoids and phenolic compounds which were documented in considerable amount in this taxon (Table 3).

Molecular Sequencing: A single band of ~600 bp was obtained on amplification of the ITS region of the fungus. The sequence of 514 bp ITS region of the fungus showed 92% identity with the *Pleurotus cystidiosus* strain ACCC 51280 ITS 1, 5.8S rRNA gene and ITS 2, complete sequence; and 28S rRNA gene, partial sequence. The phylogenetic tree constructed with the TREECON software is shown in Figure 3. In the tree, sequences of reference strain were obtained from the NCBI GenBank.

DISCUSSION

The morphological and microscopical characters along with cultural and molecular studies undertaken reveal that the present collection is typical of *P. cystidiosus* subsp. *abalonus* which is being described here from a living host for first time from India. The culture of this mushroom is characterized by formation of coremia which represents the anamorphic stage of the fungus and was described as *Antromycopsis broussonetiae* [11]. Earlier this mushroom has been reported to grow on red maple [12]. Presently, it has been found growing in caespitose clusters on living stem of *Mangifera indica*, although earlier it was documented from the dead log [2]. Han and his associates while working on a coremiopleurotus from Taiwan, described *P. abalonus* as a new species [13], however, later the anamorphic stages of *P. cystidiosus* and *P. abalonus* turned out to be belonging to the same taxon which was subsequently named and described as *Antromycopsis macrocarpa* [14].

This conclusion was based on the outcome of isozyme and molecular studies using biological material originally assigned as *P. cystidiosus* and *P. abalonus* [15]. As is the case in other edible mushrooms [8], the nutritional and nutraceutical profile of this mushroom compares equally well as it has good amount of carbohydrates, proteins, fibers, ash, minerals and substantially low amount of fats which makes it suitable vegetable for persons suffering from variety of ailments including diabetes, high blood pressure, etc. High amount of antioxidants in the mushroom accounts for its better antioxidant properties [15] which is apparent in *P. cystidiosus* subsp. *abalonus* because of the presence of ascorbic acid, carotenoids and phenolic compounds in considerable amount.

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