Journal on New Biological Reports
JNBR 6(3) 129 – 133 (2017)

ISSN 2319 – 1104 (Online)

Published by
www.researchtrend.net

Isolation, molecular characterization and qualitative screening for lignocellulolytic enzymes of *Porostereum spadiceum*: A new record of corticoid basidiomycetes from district Hamirpur (H.P.)

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| **Received**: 09 November 2017 | **Accepted**: 10 December 2017 |

ABSTRACT

Porostereum spadiceum (Pers.) Hjortstam & Ryvarden a corticoid basidiomycetes fungus has been isolated from bamboo culms collected from district Hamirpur (H.P.). It has not been reported from this district before. The identification of the isolate is confirmed by molecular characterization. The fungus exhibit both lignin modifying and cellulose degrading enzyme activity.

Key words: Corticoid, Basidiomycetes, Lignin, cellulose.

INTRODUCTION

During the survey of the district Hamirpur H.P. for basidiomycetes fungi, a species with morphological characteristics of the genus *Porostereum* collected on Bamboo culm. A comparison with the previously described species, revealed it to be *Porostereum spadiceum*. It was previously reported from India by Rattan (1977), Dargan *et al.* (2006), Sharma (2012) as *Lopharia fulva*, listed by Prasher and Ashok (2013), Dhingra *et al.* (2014) and Ritu *et al.* (2015). However it is being reported from district Hamirpur for the first time (Bilgrami *et al.* 1991, Jamaluddin *et al.* 2004). It is found in common occurrence and cause decay of important trees, therefore it has been screened for wood degrading enzymes in order to be employed in biotechnological industries.

MATERIALS AND METHODS

The collected specimen was placed in paper packets of suitable size. The field data (*viz.* collection number, details of locality, type of host/substrate, attachment of the fructification/s with host/substrate, type of forest, the date of collection and name of the collector) was placed on the packets.

The specimen was studied later in the laboratory for morphological characters. It was mounted in 3% KOH, cotton blue (in lactic acid) for determining the cyanophilous reaction, melzer's reagent (for determining the amyloidity), 1% aqueous solution of congo red and Phloxine (to determine the presence or absence of clamps and for measuring the hymenial elements and hyphae). It was studied and photographed using transmission microscope (VRS-2f) for microscopic characters.

All measurements were taken with the help of Pro MED software.

It has been isolated on Malt extract agar (Malt Extract 20g, Agar agar 20g, distilled water to make 1000ml). The stock cultures were maintained at ±4˚C. For all the experiments the fungus was sub cultured on same medium and grown on ±24˚C.

Molecular characterization

Fungal isolate was homogenized in liquid nitrogen and then DNA was extracted using the cetrimide tetradecyl trimethyl ammonium bromide (CTAB) method (Möller *et al.* 1992). The internal transcribed spacer (ITS) region of fungal rRNA genes was amplified using the universal primer set: ITS1 (50-TCCGTAGGTGAACCTGCGG-30) and ITS4 (50-TCCTCCGCTTATTGATATGC-30) (White *et al*. 1990). The 400 - 900 bp amplicon was gel eluted and the product is sequenced by Sanger's method of DNA sequencing. The sequencing results were assembled and compared with public databases Genbank (http://www.ncbi.nem.nih.gov) by using the BLAST N sequence match routines. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling 1965) and are in the units of the number of amino acid substitutions per site. The analysis involved 22 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 517 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*. 2016).

Qualitative enzyme assay

The fungus was screened for the lignocellulytic enzyme activity qualitatively. Activity of cellulase enzyme was detected by dye staining of carboxymethyl cellulose (CMC) after Pointing *et al.* (1999) and Azure-B agar clearance method was used to detect the activity of lignin modifying enzyme after Archibald (1992).

RESULTS

Taxonomy

Porostereum spadiceum (Pers.) Hjortstam & Ryvarden, Synopsis Fungorum 4 : 51, 1990. **Fig. 1**

=*Thelephora spadicea* Pers., Synopsis methodica fungorum (Göttingen) 2: 568, 1801.
=*Lopharia spadicea* (Pers.) Boidin, Bull. mens. Soc. linn. Lyon 28(7): 211, 1959.

Basidiocarps effused- reflexed occurring in resupinate form as well as membranous, adnate, often arising as small colonies which may coalesce later and become effused and reflexed, up to 1 mm thick in section; pileus flabelliform to umbonate; Upper surface brown to dark brown, tomentose, azonate to concentrically zonate, zones of erect and appressed tomentum; hymenial surface smooth, creamish white in margin, margin thin, incurved and sharp.

Hyphal system dimitic; generative hyphae 3.2-5.5 µm wide, septate, clamped, thin to thick walled, subhyalyne to tinted brown; skeletal hyphae 3.8-5.5 µm wide, unbranched, aseptate. the walls subhyaline to light brown, thick. Pseudoocystidia 43.0-88.3×3.9-5.9 µm; cylindrical, immersed or projecting out of the hymenium, the walls thick often leaving a narrow lumen, subhyaline when young but become light brown with age, unincrusted or slightly incrusted especially near the apices. The pseudoocystidia are prolongations of skeletal hyphae which run horizontally in the context and then curve in to the hymenium. These are of limited growth often with a clamp at the base and are often mistaken for a true cystidium. Basidia 15.0-30.5× 5.8-6.7 µm, Clavate, 4-sterigmata. Basidiospores 6.4- 8.0× 3.4-4.4 µm; ellipsoid, minutely apiculate, the walls hyaline, smooth, nonamyloid.

Collection Examined – India: Himachal Pradesh, Hamirpur, on Bamboo culm, Manju, 34604 (PAN), August 28, 2014.

Molecular Identification

DNA from culture was successfully extracted and amplified using the ITS primer pair. After sequencing, blast results revealed the species identity of the unknown fungi to be *Porostereum spadiceum.* The optimal tree with the sum of branch length = 12.13236708 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree **(Fig.2)**. The sequence obtained has been submitted at NCBI GenBank (accession no. MF948915).

Qualitative enzyme screening

The fungus *Porostereum spadiceum* exhibited positive cellulose degrading and lignin modifying enzyme activity. The production of purple color under and around the fungal colony was considered as a positive reaction resulting from decolorization of Azure B and the yellow opaque area against a red color of undegraded CMC indicates positive cellulolytic activity **(Fig. 3)**.

DISCUSSION

Members of class Agaricomycetes (Phylum - Basidiomycota, sub- phylum – Agaricomycotina), having hymenomycetous basidiocarps, 2-8 spored basidia and perforate to imperforate parenthesomes (Hibbett *et al.* 2007 and Kirk *et al.* 2008) are characterized as corticoid fungi. The genus *Porostereum* has characteristic effused-reflexed basidiocarp; hymenial surface smooth to

tuberculate; hyphal system dimitic; basidiospores smooth oblong. Previously it was described as *Lopharia* (Rattan 1977) now shifted to genus *Porostereum* (Hjortstam & Ryvarden 1990). *Porostereum* constitute 4 known species (*fide* index fungorum). *Porostereum spadiceum* is characterized by the presence of characteristic pseudocystidia which are encrusted apically sometimes. It was previously reported from India by Rattan (1977) from Kinnaur (H.P.), U.K., Dargan *et al*. (2006) from Chandigarh, Sharma (2012) from Kullu (H.P.) and Uttarakhand as *Lopharia fulva*, listed by Prasher and Ashok (2013) from districts Kinnaur and Kullu, Dhingra *et al.* (2014) from district Kinnaur (H.P.) and Ritu *et al.* (2015) from district Kangra (H.P.). However it is now reported from district Hamirpur for the first time (Bilgrami *et al.* 1991, Jamaluddin *et al.* 2004). Further positive results for lignocellulolytic activity indicates its potential application in biotechnological processes.

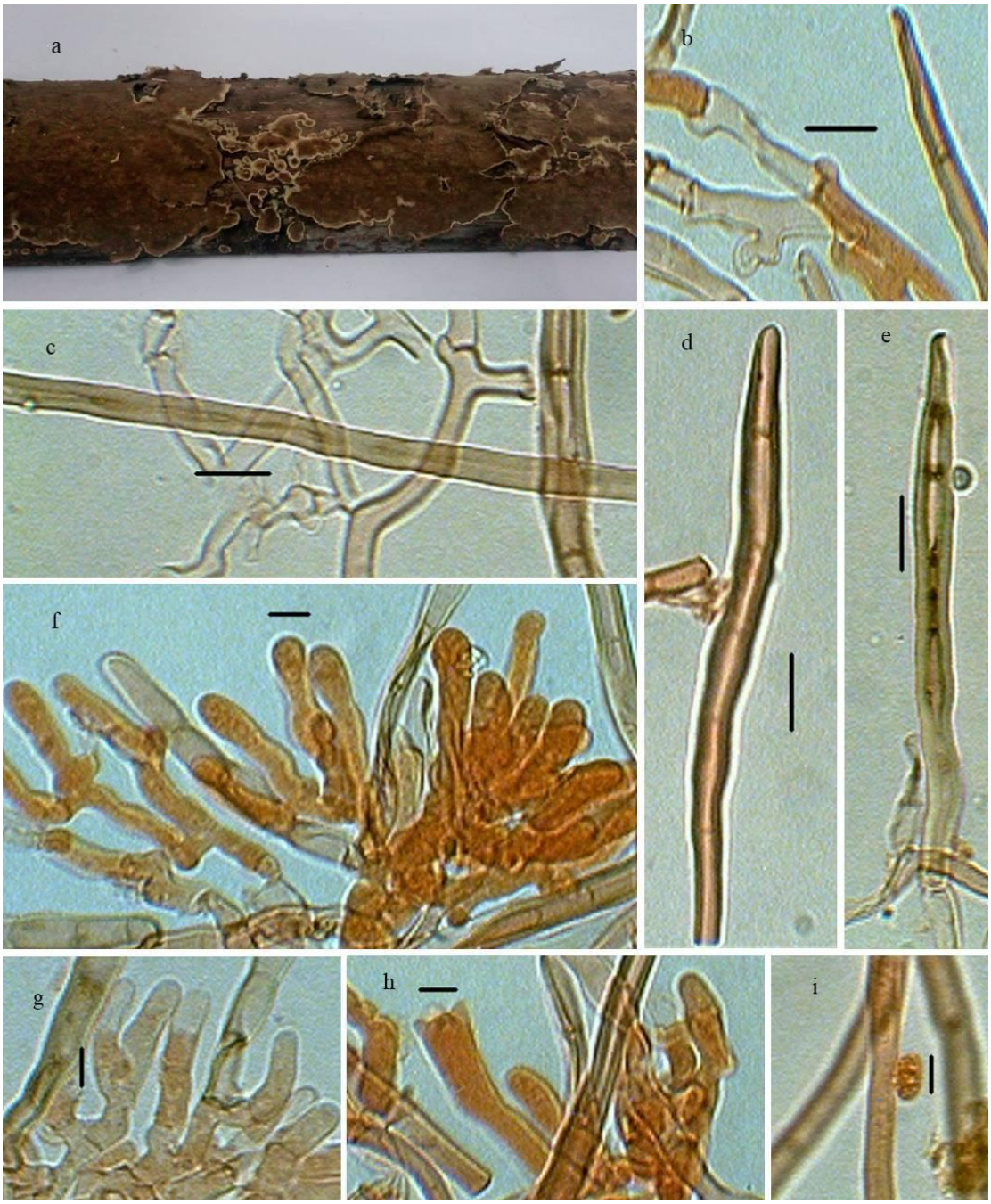

Fig. 1. ***Porostereum spadiceum.*** a) Fruit body on bamboo stick b) Generative hyphae with clamp connection c) skeletal hyphae d-e) Skeletocystidia f) Basidia g) cystidia with basal clamp h) Basidia with four sterigmata i) Basidiospore. Bars b-e = 10 µm f-i = 5 µm.

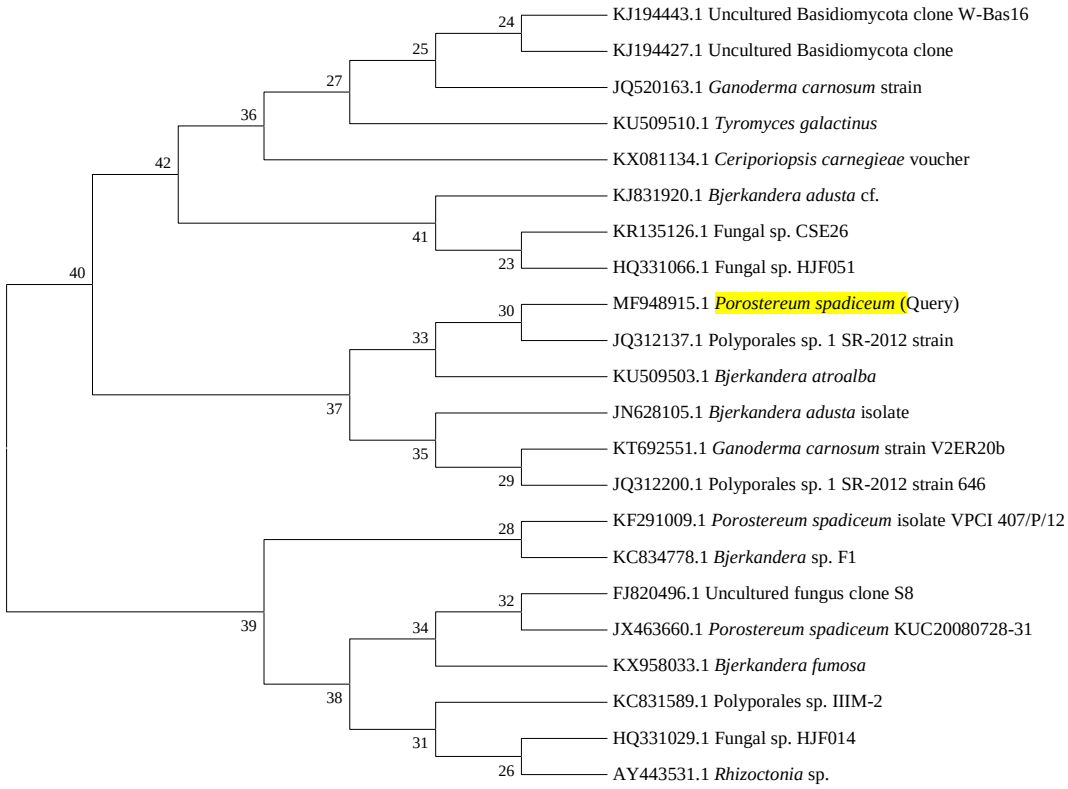

Fig. 2. Phylogenetic analysis of partial ITS rDNA gene sequence of *Porostereum spadiceum* and related microorganisms, built with the help of MEGA 7.0 software by the neighbor-joining method with Bootstrap values (1000 replicate runs).

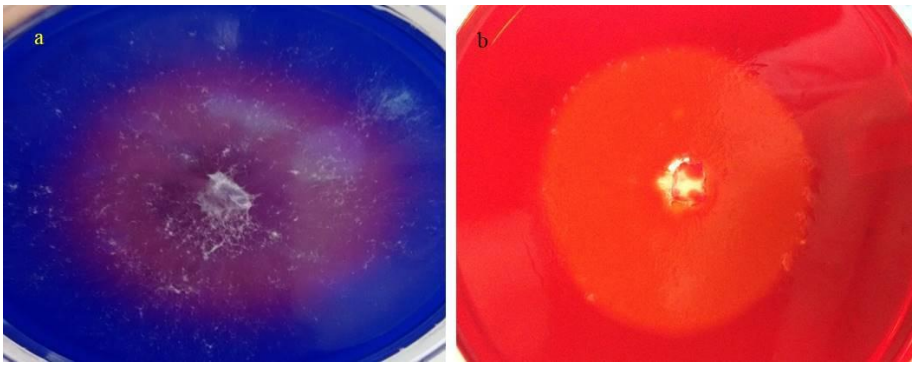

Fig. 3. a) Lignin modifying enzyme activity b) Cellulase activity

ACKNOWLEGDEMENT

The authors are thankful to University Grants Commission (SAP DRS-III) for financial support and the chairperson, Department of Botany, Panjab University, Chandigarh for infrastructural facilities.

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