



Isolation of Indigenous Brown Rot Fungi from Rotten Wood from Selected Areas of Pakistan

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Authors' contributions

This work was carried out in collaboration between all authors. Author RTM designed the study, performed the experiments, wrote the protocol and wrote the first draft of the manuscript. Authors MJA, MG and MA managed the analyses of the study. Authors DA, PA and NZ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Wood decaying fungi are very good candidate for industrial processes like; enzymes productions, dyes biodegradation and wastewater decolorization. Many studies have been conducted to explore their potential for successful industrialization. There is still need to isolate more and more new species so that these can be used for beneficial processes.

Methodology: The current study was conducted to collect Brown Rot Fungi (BRF) species from

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rotten wood and their morphological identification. The collection was made from Islamabad, Rawalpindi and Murree areas of Pakistan. Collected fungi species were cultured on Malt Extract Agar media at pH 5.5 and pure culture plates were submitted in Mycology Laboratory, Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi Pakistan for morphological identification by mycologist.

Results: More than 20 species were collected, out of these 05 were cultured on Malt Extract Agar media and identified on morphological basis. Two of these are common brown rot fungi belong to basidiomycota and three are un-common brown rot fungi belong to ascomycota.

Conclusion: Rotten wood is a very good source of brown rot fungi. Isolated BRF species can be used to explore their potential of biodegradation of textile dyes.

Keywords: Wood decaying; brown rot fungi; wastewater; decolorization; malt extract agar.

1. INTRODUCTION

Brown rot is a kind of wood decay caused by Basidiomycota, namely Agaricomycetes and some species of ascomycota. This class encompasses many orders and families, though the overwhelming majority of the BRF belongs to the *Agaricales*, *Hymenochaetales*, *Gloeophyllales* and *Polyporales*. Interestingly, only 6% of all the known wood decay fungi are now known to cause a brown rot and are almost exclusively associated with conifers [1].

Brown Rot Fungi (BRF) includes wood decaying basidiomycetes, some uncommon BRF belongs to ascomycota like *Aspergillus fumigatus* and *Aspergillus sydowii*, that are widely distributed in the world [2,3]. These fungi have the ability to remain active in extreme environmental conditions regarding the presence of chemicals. These can be used to solve various environmental issues like, wastewater treatment, toxic dyes decolorization and industrial enzymes production. Isolation of fungal species from new area will play an important role for reporting new species and support the ongoing research [4-6].

Brown Rot Fungi has non-specific enzymatic system comprising of lignin peroxidase, lignin peroxidase and laccase, having the ability of cell wall component modification and degradation [7]. Brown Rot Fungi degrade cellulose and hemicellulose while cause the modification of lignin and gave brown color to rotten wood [8]. Due to non-specific enzymatic system BRF can be actively used for the bioconversion of textile dyes having similar structure like cell wall components. Isolated BRF species can also be used for the production of extracellular enzymes which are responsible for the wood degradation, because these are industrially important enzymes [9-10].

Daedalea is a genus of fungi which was circumscribed, it is restricted to species produce brown rots and have different morphology as described by different scientists [11-12]. *Daedalea dickinsii* belong to Polyporaceae family which is an annual red rot fungus [13]. *C. puteana*, brown rot fungi belong to *Boletaceae* family also cause rot in wood [14-15]. It is mostly present on timber, moisture places and where poor ventilation. It produces cellular enzymes which has role for decomposition of classic cycles of nature [16].

From Pakistan many BRF species has been reported but not widely applied for beneficial processes [17-18].

There is further need to explore the potential areas of these microorganisms and their application for various beneficial processes. The aim of current study was to isolate potential BRF species from Rawalpindi, Islamabad and Azad Kashmir and identify these on morphological basis. Isolated species will be further used for bioremediation process in future to get maximum benefits.

2. MATERIALS AND METHODS

2.1 Study Area

Timber Market of Rawalpindi and Islamabad, surrounding forest of Rawalpindi, Islamabad and Murree. Islamabad is located at 33°26'N 73°02'E/33.43°N 73.04°E at the edge of the Pothohar Plateau at the foot of the Margalla Hills. While Rawalpindi and Murree are located in the surrounding of Islamabad. Further hilly areas and high altitude forest of Bagh Azad Kashmir were also included. The collection of BRF from natural conditions will help in the isolation of fungal species that can survive in harsh conditions.

2.2 Sample Collection

Macroscopic BRF growing on the brown rotten wood were collected, tagged, wrapped in paper and packed in plastic bags. Whole growing part of fungi collected without damage its structure, because it also help mycologist for identification. These were brought to laboratory for further processing. Further brown rotten wood samples were also collected for the isolation of fungi from the spores in the rotten wood that fall on wood during growth of fungi.

2.3 Culturing of Fungi

BRF were culture on Malt Extract Agar (MEA) media having composition (malt extract 20 g/L, agar 15 g/L, dextrose 20 g/L, peptone 3 g/L, pH 5.5) at 28°C temperature in incubator. For culturing, fungi sample were surface sterilized (70% ethanol/ 10% chlorox) and 1 mm piece shifted to media aseptically in Leminar Flow Hood [19-20].

2.4 Identification of Fungi

Most of the fungus were macroscopic and could be identified by specialist mycologist. Those species culture on media plates, their slides were prepared and their identification was confirmed on basis of spores shape, size, texture and shape of fungal hyphae by mycologist [21].

3. RESULTS

3.1 Fungal Species Collected

More than 20 various fungi species associated with wood rotting were collected from Rawalpindi, Islamabad, Murree and Bagh Azad Kashmir. These were brought to Industrial & Environmental Biotechnology Laboratory Department of Biochemistry, PMAS-Arid Agriculture University, Rawalpindi and cultured on Malt Extract Agar media after surface sterilization of fungi (macroscopic).

3.2 Species Collected from Rawalpindi and Islamabad

The sites for the collection of Brown rot fungi from Rawalpindi and Islamabad are Small forests of Shakarparian, surroundings of Rawal Lake and Timber market of Rawalpindi. Following species were collected from these areas.

Coniophora puteana IEBL-1 was collected from Shakarparian (SP) Islamabad on the dead trunk

of *Ficus carica* (Fig. plant) on 24-11-2013. It was initially whitish in color with little shade of brown growing margins (Figs. 1a and b). Wood was converted into brown patches where fungus showed active growth. It was cultured on malt extract agar media at 28°C having pH 5, growth was clear after 96 hours (Fig. 1c). The culture produce white mycelia which turn brownish at margins after 5 days of growth and spread on whole petri plate (Table 1). *Coniophora* is a genus of fungi of *Boletaceae* family with 20 known species with wide distribution. One notable member of the cellular fungus is *Coniophora puteana* which causes brown rot in wood [15]. This causes a darkening of the timber (brown rot) and rarely found in building, cause decay with high moisture content. The fungi secrete extracellular enzymes which break down potential food sources, which are then absorbed back into the fungal colony [16]. These have the potential to damage standing timber, finished wood products, fibers, and wide range of non-cellulosic products such as plastics, fuels, paints, glues and drugs. These enzymes include amylases, glucoamylases, oxidases, lipases, pectinases, and proteases [22].

Daedalea dickinsii IEBL-2 was collected from Chakshahzad (CS) and Lake View Park Islamabad on the dead trunk of *Ficus carica* on 14-11-2013. When collected, it was light white from lower side and brownish from upper surface (Figs. 2a and 2b) and was labeled as CS-2 and LV. Upper side has soft surface with lines at its surface and has brown color with whitish lines (Table 1). Its culture on Malt extract agar showed distinctive growth on fourth day with dirty brownish color mycelia which are thread like (Fig. 2c) (Table 1). *Daedalea* genus is restricted to species that produce brown rots and have basidiocarps with trimatrichyphal systems, clamped generative hyphae, cork-colored context and thick dissepiments apices [11-12]. *Daedalea dickinsii* belong to Polyporaceae family, which known as a red rot fungus [13,23-24].

3.3 Un-common Brown Rot Fungi

Some of the un-common Brown Rot Fungi usually consider as soft fungi, belongs to ascomycota division, were also isolated from the dead brown rotten wood. These fungi play vital role in the decay of the wood due to their enzyme system. These include the species of *Aspergillus* genus like, *Aspergillus sydowii*, *Aspergillus niger*, *Aspergillus fumigatus*.

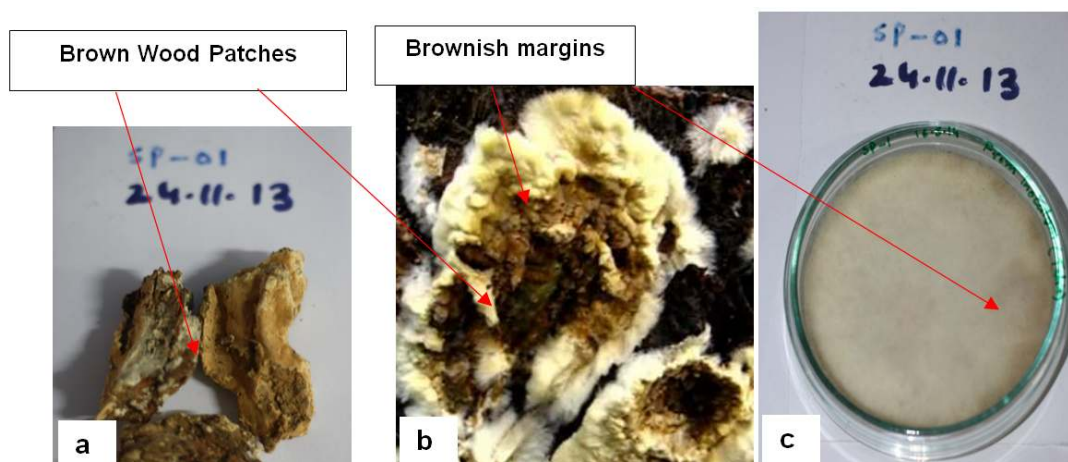


Fig. 1. *Coniphora puteana* IEBL-1, a brown rot fungi, isolated from Shakarparian Islamabad (a)- Dry sample of *Coniphora puteana* after collection (b)- Growing *Coniphora puteana* IEBL-01on rotten wood (c)- Five days old culture of *Coniphora puteana* on Malt extract agar



Fig. 2. *Daedalea dickinsii* IEBL-2, a brown rot fungi collected from chackshahzad Islamabad: (a)- fungi on dead trunk of rotten wood (b)- *Daedalea dickinsii* IEBL-2 upper and lower side after collection (c)- Culture of *Daedalea dickinsii* maintain on Malt extract agar

***Aspergillus sydowii* IEBL-5** has worldwide distribution however may occur with increased latitude, primarily found in soil and rotten wood. Fungus growth rate is moderate color is influenced by media, described as a blue-green to dark-green. *A. sydowii* produces long conidiophore stipes upto 200 um which can give the colony that woolly or hairy appearance. It was the agent of invasive aspergillosis, keratomycosis (infection of cornea) and onychomycosis (nail infection). Ein-Gil in 2009 found that *A. Sydowii* collected from marine sponges which were the reservoirs of a potential marine pathogen [25]. It has wide range of non-specific extracellular enzymes that can be used for various useful processes like; food manufacturing, clothe industry and dyes biodegradation. In current study it was isolated from brown rotten wood of pines tree (*Pinus wallichiana*) from Murree region

(Fig. 3). Its spores are light green in color with turning brownish at margins. It gave more sticky texture with media. Due to potential enzymatic system it will be used for the decolorization of textile dyes.

***Aspergillus niger* IEBL-6** is one of the most common species of the genus *Aspergillus* causes black mold disease in certain fruits, vegetables and cause rotting of timber [26]. It produces various extracellular enzymes with high efficiency, its lignolytic enzyme system can be used for decolorization of textile wastewater. It was isolated from rotted wood of *Pinus tree (Pinus wallichiana)*. Fungus was cultured successfully on Malt Extract Agar media at 28°C, brown colonies also showing green patches. It has powder like texture and spores spread easily in air (Fig. 4).

Table 1. Morphological characteristics of brown rot fungi IEBL-1 and IEBL-2

| Fungi | Location | Plant | Plant status | Fungal cap/ Fruit body | Pores | Flesh of culture | Spores | Habitat |
|----------------------------------|-------------------------|---------------------|-----------------------|---|--|---|--|---|
| <i>Coniophora puteana</i> IEBL-1 | Shakrparian Islamabad | <i>Ficus carica</i> | Dead trunk/live plant | Brown to black in color, flat, thin plate like structure 2-3 mm thick, initially white and delicate, brittle when dry | | Initially white but later turn brown, fibrous margins, firmly attached, fragile when dry, hyphal texture loose to dense, hyphae distinct and inflated | Yellow brown, fusiform or citriform, smooth with a double wall | 50-60% humidity, attack timber wood, mostly cause internal damage and leave thin skin of wood |
| <i>Daedalea dickinsii</i> IEBL-2 | Checkshahzad, Islamabad | <i>Ficus carica</i> | Dead/ live plant | Shelf- to hoof-like and brown, 20 cm long, 10 cm wide, 5 cm thick, margin usually dull, flesh very tough, hard, sessile, dimidiate (grow in layers) | Circular to angular, some elongated, 1-2 mm, tubes up to 1.5 cm deep, pinkish buff, 2 cm thick | Hyphal system trimitic, brownish skeletal, not inflated and thin walled | Deposited, whitish cylindrical, thin walled, single celled | At moist places, on cellulosic materials, damage the live and dead woody plants |

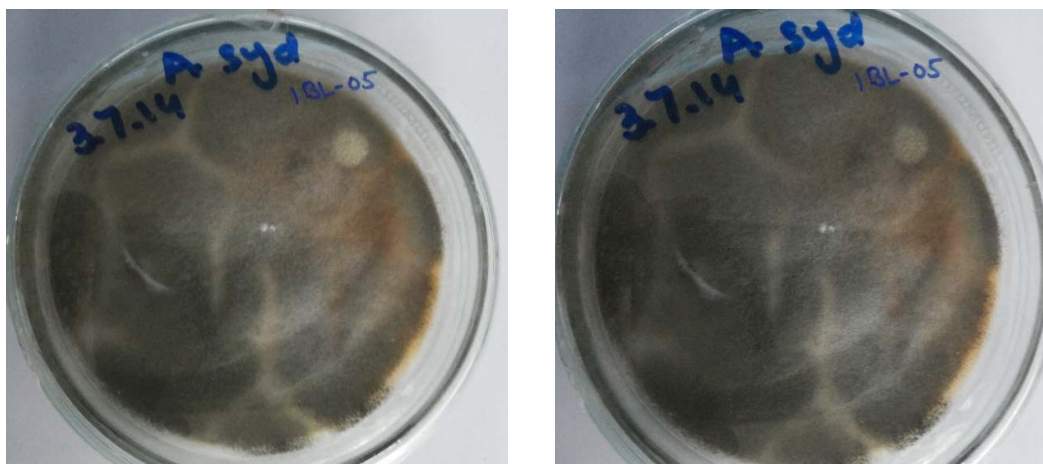


Fig. 3. *Aspergillus sydowii* IEBL-5, isolated from rotten wood of pinus (*Pinus wallichiana*) cultured on malt extract agar media

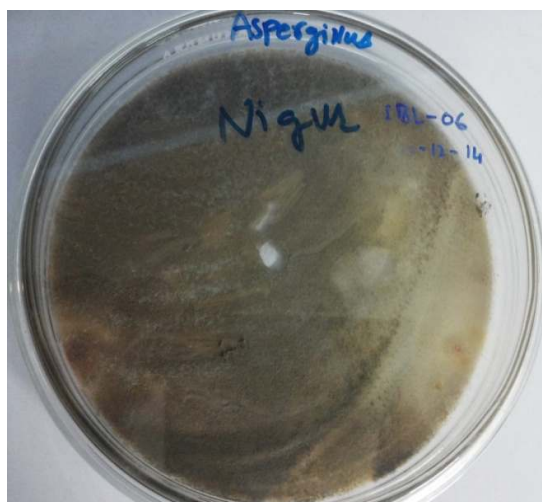


Fig. 4. *Aspergillus niger* IEBL-6, isolated from rotten wood of *Pinus wallichiana* collected from timber market of Rawalpindi

***Aspergillus fumigatus* IEBL-7** is one of the most ubiquitous of the airborne saprophytic fungi. *A. fumigatus* plays an essential role in recycling environmental carbon and nitrogen [27]. Its natural ecological niche is the soil, wherein it survives and grows on organic debris. Although this species is not the most prevalent fungus in the world, it is one of the most ubiquitous of those with airborne conidia. *A. fumigatus* has enzymes as (lactate dehydrogenase, superoxide dismutase, isocitrate dehydrogenase, aspartate aminotransferase, glucose-6-phosphate dehydrogenase, and phosphogluconate dehydrogenase) have been reported to be monomorphic, although data vary from study to

study, and other enzymes (malate dehydrogenase, glucose phosphate isomerase, phosphoglucomutase, hexokinase, esterase, malate dehydrogenase, peptidases, fructose kinase, purine nucleoside phosphorylase, and phosphatases) display polymorphic patterns. [28]. The isolated fungus has light green color with powdery texture on MEA media (Fig. 5). It was obtained from the dead trunk of *Pinus roxiburghii* which was collected from Murree. Its culture has powdery texture and spores distribute if exposed to air. It needs to culture with great care to avoid its contamination in other cultures.

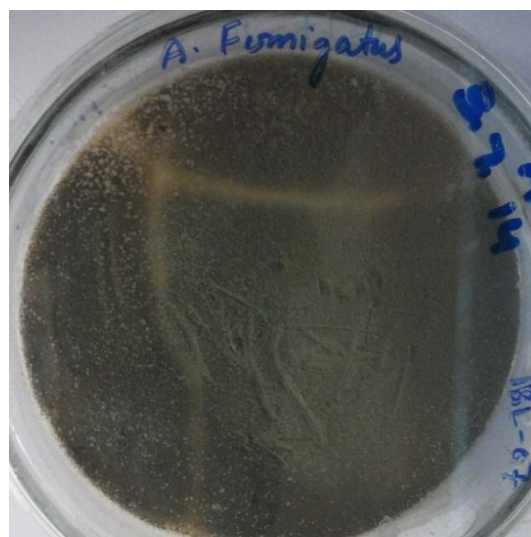


Fig. 5. *Aspergillus fumigatus* IEBL-7 isolated from rotten dead trunk of *Pinus roxiburghii* collected from Murree region of Pakistan

4. DISCUSSION

Fungi are the microorganisms with thousands of species found throughout the world. Most of these are beneficial for human being in many different ways. There is further need to explore their beneficial processes and to find the ways to utilize these processes in efficient ways. Hundreds of microorganisms are used for different processes but there is still need to explore more beneficial microorganisms. These are distributed in various localities, there is further need to explore the potential areas of these microorganisms. The current study was to isolated potential BRF species from Rawalpindi, Islamabad and Murree and identifies these on morphological basis. These BRF species will be further use for bioremediation process in future to reduce environmental pollution problems.

5. CONCLUSION

More than twenty fungi have been collected during current study from rotting wood (brown color) and five were successfully cultured on Malt extract Agar media. This suggested that MEA media is suitable for the growth of brown rot fungi. The most suitable growing temperature for these fungi is 28°C and suitable pH 5.5. Rotting wood is very good source of microbes involved in rotting. These microbes could be used for other beneficial processes after identification like production of enzymes and biodegradation of wastewater.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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