Isolation of Indigenous Brown Rot Fungi from Rotten Wood from selected areas of Pakistan

Abstract
Wood decaying fungi are very good candidate for industrial processes like; enzymes productions, dyes biodegradation and wastewater decolorization. Many studies has been conducted to explore their potential for industrialization. There is till need to isolate more and more new species so that these can be used for beneficial processes. The current study was conducted to collected Brown Rot Fungi (BRF) species from rotten wood, their identification and culturing on various media. These were collected from Islamabad, Rawalpindi and Murree. More than 20 various species were collected, 05 were cultured on Malt Extract Agar media and identified on morphological basis by mycologist. Out of these two are common brown rot fungi and three are un-common brown rot fungi. Isolated species will be used to explore their potential of biodegradation of textile dyes.

Key Words: Wood decaying, Brown Rot Fungi, wastewater, decolorization, Malt extract agar

Introduction
Brown rot is a kind of wood decay caused exclusively by Basidiomycota, namely Agaricomycetes. 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 (Hibbett and Donoghue, 2001).
Brown Rot Fungi (BRF) includes wood decaying basidiomycetes that are widely distributed in the world (Dutton and Evans, 1986; Shimada et al., 1997). These fungi has the ability to remain active in extreme environmental conditions regarding the presence of chemicals. These can used to solve various environmental issues like, wastewater treatment, toxic dyes decolorization and industrial enzymes production. Isolation of fungal species form new area will play important role for reporting new species and support the ongoing research (Bertea and Bertea, 2008; Bisschops and Spanjers, 2003; Carliell et al., 1994).
Brown Rot Fungi has non-specific enzymatic system comprising of maganase peroxidase, lignin peroxidase and laccase, having the ability of cell wall component modification and degradation (Mai et al., 2004). Brown Rot Fungi degrade cellulose and hemicellulose while cause the modification of lignin and gave brown color to rotten wood (Kirk and Cullen, 1998). 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 these extracellular enzymes which are responsIEBLe for the wood degradation, because these are industrially important enzymes (Kumar et al., 2008; Hammel et al., 2002).
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 scientist (Ryvarden, 1991; Nunez and Ryvarden, 2001). Daedalea dickinsii belong to Polyporaceae family which is an annual red rot fungus (Bae and Min, 2000). The other brown rot fungi as Coniophora genus which belong to Boletaceae family have species C. puteana cause rot in wood (Ginns, 1982; Kirk et al., 2008).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(Carroll and Wicklow, 1992).
From Pakistan many BRF species has been reported but not widely applied for beneficial processes (Ambrosio and Campos, 2004; Sumathi and Manju, 2000). There is further need to explore the potential areas of these microorganisms and their application for various beneficial processes. The aims of current study is to isolate potential BRF species from Rawalpindi, Islamabad and Azad Kashmir and identify these on morphological basis. Isolated species will be further use for bioremediation process in future to get maximum benefits.

Materials and Methods

Study Area: Timber Market of Rawalpindi and Islamabad, surrounding forest of Rawalpindi, Islamabad and Murree. 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.

Sample Collection: Macroscopic BRF growing on the brown rotten wood were collected, tagged, rapped in paper and packed in plastic bags. 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.

Culturing of Fungi: BRF were culture on Malt Extract Agar (MEA) media having composition (malt extract 20g/L, agar 15g/L, dextrose 20g/L, peptone 3g/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.

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 (Krieger 1967).

Potential BRF species: Cultured BRF species are potential agent for the production of extracellular enzymes for industrial processes and for the bioremediation process of textile industrial effluent and textile dyes. Isolated BRF species will be used for textile dyes biodegradation in future.

Results

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) or rotten wood.

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 Timmer market of Rawalpindi. Following species were collected from these areas.

*Coniophora puteania 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 (Fig.1a and b). Wood was converted into brown patches where fungal 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. *Coniophora* is a genus of fungi of Boletaceae family with 20 known species with wide distributionone notable member of the cellular fungus is *Coniophora puteana* which causes brown rot in wood (Kirk et al., 2008). 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 (Carroll and Wicklow, 1992). 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 (Berka et al., 1992).

*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 (Fig. 2a and 2b) and labelled as CS-2. Upper side has soft surface with lines at its surface and have brown color with whitish lines. Its culture on Malt extract agar showed distinctive growth on fourth day with dirty brownish color mycelia which are thread like (Fig. 2c). *Daedalea* genus is restricted to species that produce brown rots and have basidiocarps with trimatrichyphal systems, clamped generative hyphae, cork-coloured context and thick dissepiments apices (Ryvarden, 1991, Nunez and Ryvarden, 2001). *Daedalea dickinsii* belong to Polyporaceae family, which known as a red rot fungus (Bae and Min 2000; Selbmann et al., 2002; Silvi et al., 2013).

![Brown Wood Patches](image1)

![Brownish margins](image2)

![Culture](image3)

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

Some of the un-common Brown Rot Fungi usually consider as soft fungi 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.

*Aspergillus sydowii* IEBL-5 has worldwide distribution however may occure 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 up to 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 (Ein-Gil et al., 2009). 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.

![Fig. 3: Aspergillus sydowii IEBL-5, isolated from rotten wood of pinus (*Pinus wallichiana*) cultured on Malt extract agar media](image)

*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 rottening of timber (Samson et al., 2001). 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).

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

*Aspergillus fumigates* 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 (Haines, 1995). 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. (Chazalet et al., 1998). The isolated fungus has light green color with powdery texture on MEA media (Fig. 5). It was obtained from the dead trunk of *Pinus roxburghii* which was collected from Murree. Its culture has powdery texture and spores distribute if exposed to air. It need to culture with great care to avid its contamination in other cultures.
Fig. 5: *Aspergillus fumigates* IEBL-7 isolated from rotten dead trunk of *Pinus roxiburghii* collected from Murree region of Pakistan

**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 process but there is need to explore more and more beneficial microorganisms. These are distributed in various localities, there is further need to explore the potential areas of these microorganisms and their application for various beneficial processes. The current study was to isolated potential BRF species from Rawalpindi, Islamabad and Murree and identify these on morphological basis. These BRF species will be further use for bioremediation process in future to reduce environmental pollution problems.

**References**


