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Identification of Wood Rot Fungi in the Historic Baker Memorial Hall at the University of the Philippines, Los Baños Campus

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Deterioration in heritage wood structures caused by wood-decay fungi is a worldwide concern. In the University of the Philippines Los Baños, decay fungi from wooden canopies of the Baker Memorial Hall were identified and its degrading ability was evaluated. Specimen collection was conducted on degraded canopies made of *Mayapis* (*Shorea palosapis*) with signs of white or green fungal growth. Fungi associated with the rotting symptoms were isolated, purified, characterized and identified. Colonies of fungal isolates were fast-growing in malt extract agar (MEA), with colony diameter reaching 5.60 ± 0.43 cm (WRF8) and 5.15 ± 0.25 cm (WRF5) after a day. Mycelia of isolates were hyaline and rhizoidal on water agar. Isolate WRF5 produced green, raised colonies that imparted yellow pigmentation on potato dextrose agar (PDA) and MEA. Generally, colonies were raised, radially striated with green center and white margin on PDA. Average length and width of WRF5 conidia were 3.19 ± 0.33 µm and 2.73 ± 0.36 µm, respectively. Cross-referencing morphological and cultural data with related literatures and identification keys indicated the isolated fungi were *Trichoderma viride* (WRF1), *T. crassum* (WRF3), *T. reesei* (WRF5), *Rhizopus* sp. (WRF8) and *Coniophora* sp. Furthermore, inoculation of 10^8 *T. reesei* conidia/mL on clean *Mayapis* wood blocks resulted to profuse fungal growth, with average weight loss recorded at 8.81 ± 0.79% at 8 wks and 10.53 ± 0.88% at 14 wks which indicated a considerable but slow wood degradation**.**

Keywords: wood, biodeterioration, wood rot, heritage building, *Trichoderma*

INTRODUCTION

In the forest ecosystems, the role of fungi is most often symbiotic or saprophytic to trees. Although some maybe pathogenic, most mushrooms and other fungi also serve as natural decomposers of wood in the forest, thus are beneficial for nutrient cycling. However, problems arise as man started to commercialize wood because these organisms attack infrastructures and wood products out of their natural habitat. Wood is a popular material since the ancient times because of its ease of processing with simple tools and natural availability. Filipino ancestors used wood in transporting and housing the living with *barotos* (dugout boats), *balangays* (plank-built boats), *bale* (Ifugao house) and

torogans (Maranao house), and the dead with *kabaongs* (coffins) (Fadriquela, 2013).

However, susceptibility of wood to detrimental factors such as weather, moisture, fire, pests and biological degradation threatens infrastructures, especially heritage wooden structures and artifacts. Wood conservators are particularly concerned with the effect of biological degradation, because it results to slower but greater damage. Termites and wood degrading microorganisms are some of the pests causing serious damage (Unger et al., 2001). Wood-rotting microorganisms include many species of fungi that secrete enzymes such as cellulase, hemicellulase, lignin-peroxidase, manganese-peroxidase, etc. which degrade wood structures down to its monomeric units (Deacon, 2005; Schmidt, 2006; Schmidt, 2007). Depending on its mode of action and physical appearance, wood rot may be classified as brown rot, white rot or soft rot. Occurrence of wood rot usually requires high relative humidity (RH) and moderately low temperature and is caused by a number of Basidiomycota and some Ascomycota species. Soft rot fungi attack wood with low lignin content, producing unique holes in the secondary cell wall layers (Schmidt, 2006). White rot is caused by fungi that attack lignin, cellulose and hemicellulose components while brown rot is caused by fungi that only attack cellulose (Ochoa, 1978; Schmidt, 2006).

The main goal of this study was to identify the fungi causing the degradation of wood components in Charles Fuller Baker Memorial Hall (or Baker Hall) in the University of the Philippines Los Baños, Laguna, Philippines. This heritage structure is one of the oldest buildings in the campus and it was named after Charles Fuller Baker who was a Dean in the university from 1918 to 1927 (IRRI, 2000). It was declared as a historical site by National Historical Commission of the Philippines in 2015. It served as former internment camp of prisoners from Allied countries during World War II. Some physical education courses are being held here but the university plans to transform it into a Cultural Hub and Recreation Center to showcase its rich historical heritage, while still preserving this old wooden built heritage (UPLB OVCCA, 2015).

Wood deterioration is caused by fungi (Morris and Winandy, 2002), but some are caused by bacteria and insects. Studies are extensively conducted to find various ways of conserving and managing biological degradation of wood artifacts and infrastructures. In the Philippines, Giron et al., (1982) conducted a research on fungi causing soft rot of wood samples from portions of cooling towers, railway ties and electric posts in provinces of Mindoro, Cebu, Bacolod and Dumaguete. Hyphal fragments of *Fusarium moniliforme* (Sheldon), *Diplodia theobromae* ((Pat.) W. Nowell), *Curvularia inaequalis* ((Shear) Boedijn), *Trichoderma* sp. (Pers.), *Humicola grisea* (Traaen) and *Aspergillus* sp. (P. Micheli ex Haller) were identified in the samples. Moreover, another related study was conducted to identify macroscopically wood-rotting basidiomycetes in the Molave forest in San Fernando City, La Union. Fifty-one species from the genera *Ganoderma* (P. Karst.), *Entoloma* (Fr. ex P. Kumm.), *Lenzites* (Fr.) and *Laetiporus* (Murrill) were identified (Tadiosa and Arsenio, 2014).

Specimen collection done at Mt. Palaypalay-Mataas na Gulod Protected Landscape in the municipalities of Ternate and Maragondon in Cavite and Nasugbu in Batangas yielded 59 fungal species belonging to Basidiomycota and Ascomycota. Some fungal species identified to cause wood rot in this study includes *Auricularia* (Bull. ex Juss.), *Ganoderma* (P. Karst.), *Trametes* (Fr.), *Phellinus* (Quél.), *Entoloma* (Fr. ex P. Kumm.), *Armillaria* ((Fr.) Staude), etc. (Capon-Arenas et al., 2015). Fungal species identified by Ochoa (1978) associated with wood rot in forest litter included two Trichoderma isolates. In the study, isolates were grown on MEA and microscopic examination and cultural characterization of each isolate were conducted. Similarly, Hui et al., (2013) conducted cultural characterization of various isolates of Trichoderma grown on PDA and identified up to the species level using DNA sequence analysis.

The study of Błaszczyk et al., (2014, 2016) that focused on the characterization of a number of Trichoderma species according to their genetic diversity and in terms of xylanolytic and cellulolytic activities identified the presence of *Trichoderma atroviride* (Karst.), *T. citrinoviride* (Bissett), *T. cremeum* (Chav. and Samuels), *T. gamsii* (Samuels and Druzhin), *T. harzianum* complex, *T. koningii* (Oudem), *T. koningiopsis* (Samuels *et al*.), *T. longibrachiatum* (Rifai), *T. longipile* (Bissett), *Trichoderma* sp, *T. viride* (Schumach) and *T. viridescens* complex. The most abundant fungus was *T. viride*, that was observed in 53% of the samples from different locations in Central Europe. The result of the experiment implies *Trichoderma* spp. also may have the ability to cause soft rot.

Wood decay fungi attack many species of

wood. In this study, *Shorea palosapis* (Blanco) Merr., locally known as *Mayapis*, was identified as the material used in the window canopies of Baker Hall from where the fungal isolates were obtained. *Mayapis* belongs to the family *Dipterocarpaceae*, class *Magnoliopsida*. International timber market is predominated with dipterocarp wood species. Thus, it is important for Southeast Asian countries with dipterocarp forests to protect the trees from pests and pathogens that attack it to secure its economic gains, not just to preserve heritage infrastructures (Appanah et al., 1998).

MATERIALS AND METHODS

Preparation of Growing Media and Wood Blocks.

Potato dextrose agar (PDA) and malt extract agar (MEA) were used as the artificial media in the evaluation of growth characteristics of the fungi associated with wood rot. To prepare PDA, potato water is prepared by boiling 200 g of peeled potato in 500 mL of distilled water. Potato water was then added with distilled water to obtain 1 L final volume, which was added with 20 g dextrose and 20 g agar. To prepare MEA, 20 g of malt extract was mixed with 20 g of agar in 1 L of distilled water. These mixtures were boiled using microwave oven, then dispensed in heat-resistant bottles prior to sterilization at 121°C and 15 psi for 15 min in a pressure cooker. Moreover, to prepare the wood blocks, 3 cm x 3 cm x 3 cm blocks were cut in a larger piece of *Mayapis* wood. The cutting was done in the wood workshop at the Department of Forest Products and Paper Science at the College of Forestry and Natural Resources, UPLB. The wood blocks were sterilized at 121°C and 15 psi for 15 min in a pressure cooker, prior to use in the *in vitro* wood degradation test.

Isolation of wood rot-associated fungi.

Moist sampling using sterile damp cotton and dry sampling using *MycoTape*™ (Mycoteam™, Forkningsveien, Oslo, Norway) were performed to obtain samples from four sides of Baker Hall. Figure 1 shows the bird's eye view sketch of the sampling sites (*i.e.* front, right (east) side, left (west) side and back). Three relative humidity and ambient temperature readings were recorded for each site to check for microclimate variation. Detached or fallen rotten wood portions of the window canopies were also collected and incubated in a moist chamber for one week to grow out the wood rot fungi. Samples from the cotton swab and a (0.5 x 0.5) cm^2 portion of *MycoTape*™ were mounted onto potato dextrose agar (PDA) plates and incubated at room temperature. Mycelial disks (0.8 cm diameter) were obtained from the PDA plates after 24 hrs and transferred to PDA, malt extract agar (MEA) and water agar (WA) for cultural characterization. MEA was used for possible detection of Basidiomycota since water agar was used to induce production of survival structures of the isolated fungus. Lastly, fungal colonies in PDA slants were refrigerated for storage and future use.

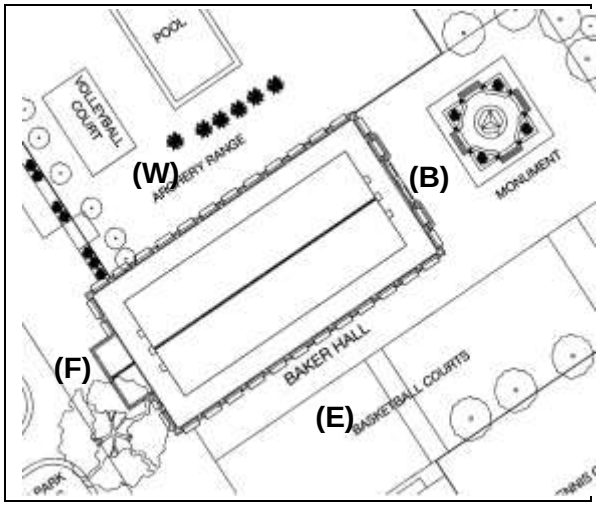

Figure 1. Bird's eyeview of the historic Baker Hall at the University of the Philippines Los Baños, Laguna, showing the sampling sites of the study. (F) front side, (E) east/right side, (W) west/left side and (B) back side. Structures pointed at by the red arrow are the wooden canopies where the random samples were obtained.

Colony and morphological characterization and Identification of fungal isolates.

Pure colonies of the isolate WFR1, WRF3, WRF5 and WRF8 were characterized after seven days of incubation. Colony characteristics of fungal isolates were described based on color, margin, elevation, form, surface appearance of its colony and pigmentation imparted on the different culture media (*i.e.* PDA, MEA, WA). Moreover, diameters of 24-hr old fungal colonies were measured in centimeters to determine size of

growth. For morphometric measurements, the length and width of 20 random conidia of WRF5 isolate were recorded using calibrated microscopes. Identification of the isolated fungi was based mainly on the colony and morphological characteristics recorded in this study with cross reference to existing literatures and online databases (Nobles, 1948; Simmons, 1977; Kubicek and Harman, 2002; Schmidt, 2002; Cumagun et al., 2009; Shrestha et al., 2009; Schmidt, 2012).

***In vitro* wood degradation test.**

A seven-day-old pure culture of WRF5 isolate in PDA was used for preparation of stock conidia suspension. Ten milliliters of sterile distilled water were used to aseptically flush and suspend the conidia. After which a serial dilution up to 10^{-3} was prepared and the average number of conidia was determined using a hemacytometer. Concentration of the stock suspension was calculated, and an aliquot was obtained to make a standardized 10^8 conidia/mL suspension. The suspension was inoculated in pre-weighed sterile wood blocks previously incubated at 50°C for 8 hrs. Three replicates for each time point were prepared and aseptically placed inside a 250 mL Erlenmeyer flask moist chamber with wet cotton inside. The setup was monitored for 8 weeks and 14 weeks, after which the blocks were oven-dried at 50°C for 8 hrs to get the final weight. Average percent weight loss of the inoculated wood blocks and the control were calculated, and the fruiting bodies of the inoculated fungus were observed under a dissecting microscope for re-association with the wood rot (Adaskaveg et al., 1991).

RESULTS AND DISCUSSION

Relative humidity and temperature affect incidence of wood rot fungi.

Ambient temperature and the relative humidity during sample collection were recorded to evaluate microclimate variation in the sampling site. Table 1 shows the average AT and RH in the four sides of UPLB Baker Hall. Sample collection was conducted on January 2018 in the morning when torrential rains occurred the night before. The average readings revealed that the back side had the lowest AT and highest RH while the right side had the highest AT and lowest RH. Fungal growth on wood was highly favored in the back side and similarly, in the front while less favored in the right and left side. Lastly, the data and fungal growth observation imply that RH influences the fungal growth more than the AT. The results also indicate that the management of wood rot fungi must focus on the front and back of the hall.

Table 1; Average ambient temperature and relative humidity of the four sides of UPLB Baker Hall during sampling of wood rot fungi.

Site	AT (°C)*	RH (%)**
Front	28.0^{b}	65.7^{ab}
East (Right)	27.8^{c}	64.7^{b}
West (Left)	28.2^{a}	64.0^{b}
Back	27.4^{d}	67.7^{a}

Note: AT: ambient temperature, RH: relative humidity. Means within the same column with the same letter superscript are not significantly different at *P<0.0001 and **P<0.05 (Tukey's Test).

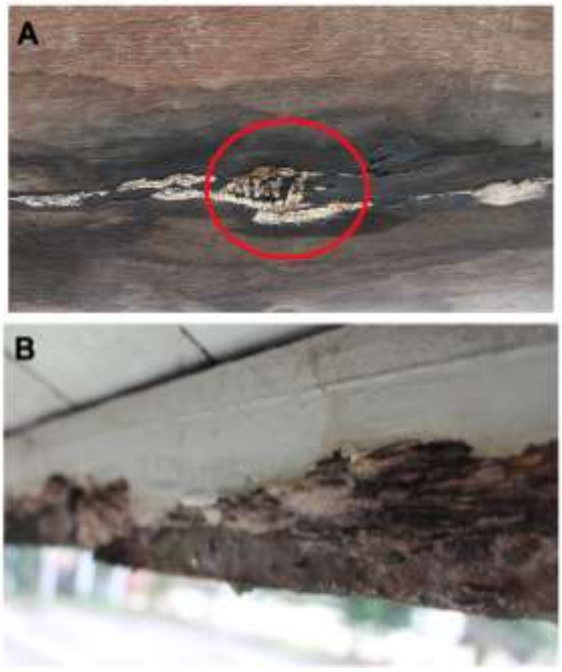

Figure 2. On-site photos of wood decay. A: Signs and B: symptoms caused by wood rotting fungus observed in UPLB Baker Hall. Encircled fungal growth was identified as *Coniophora* sp. (Phylum Basidiomycota).

On-site diagnosis and sporulation test identified *Coniophora* sp. (Basidiomycota) and a few Ascomycota associated with wood rot.

Association of the symptoms exhibited by the host and the suspected pathogen is the first step

in Koch's postulate. Figure 2 and Figure 3 show examples of the wood rot observed at the façade of the hall. The signs (Figure 2-A) of fungal growth were observed as white to cream grainy growth emanating from the margin of the decaying part of the wood. This fungal outgrowth was identified as fruiting bodies of *Coniophora* sp. (De Candolle) (De Candolle, 1815; Nobles, 1948). On the other hand, symptoms (Figure 2-B) associated with the degradation is browning of the affected part, indicative of lignin leftover after cellulosic degradation. The texture of the rotting portion is soft rather than brittle or hard, which is a characteristic of soft rot of wood, as shown in Figure 3. Moreover, sporulation test was done in a moisture chamber to favor the growth of the fungi present in the collected wood samples.

Figure 3 shows the representative wood samples after seven days of sporulation test. Green, white, yellow and black fungal structures were observed, wherein green (*Trichoderma* sp.) and black (*Rhizopus* sp. (Ehrenb.)) are the most predominant fungal colony colors.

Colony growth characteristics affirmed that *Trichoderma* spp. is the predominant fungi associated with wood rot of Baker Hall wood structures.

Colonies of the isolated fungi were characterized in PDA, MEA and WA. Figure 4-A shows the characteristics of a seven-day-old pure colony of WRF1 on PDA. Growth was raised, radially striated with flat compact tufts of green colonies and white mycelial margin. Colony size was recorded at 3.95 ± 0.17 cm in PDA and 5.00 ± 0.19 cm in MEA after a day. In PDA, the reverse side of the culture has no color. The colony characteristics of WRF 1 led to its identification as *Trichoderma viride* Pers. (Persson, 1794; Stranks, 1970; Gams and Bissett, 2002).

On the other hand, Figure 4-B shows the pure colony of WRF3 when grown on PDA after seven days. Colonies were radially striated, raised, forming compacted green colony pustules and white mycelial margin. Colony size was recorded at 4.98 ± 0.08 cm in PDA and 4.93 ± 0.08 cm in MEA after a day. Likewise, no pigmentation was observed only on MEA. The colony characteristics of WRF 1 led to its identification as *Trichoderma crassum* Bissett. (Bissett, 1991; Gams and Bissett, 2002). Figure 4-C shows the colony of WRF5 on PDA after seven days of incubation. The colony was flat, pale green scattered tufts, predominantly on a white mycelial mat. Colony size was recorded at 4.65 ± 0.09 cm in PDA and 5.15 ± 0.25 cm in MEA after a day. Moreover, yellowish pigmentation was observed both in PDA and MEA. The aforementioned characteristics collectively suggests that isolate WRF5 is *Trichoderma reesei* Simmons (Simmons, 1977; Medve *et al*., 1998; Gams and Bissett, 2002; Martinez *et al.,* 2008). Lastly, the colony of isolate WRF8 was also characterized in PDA. Figure 4-D shows that WRF8 produced thick and white mycelia with black, circular distinct structures, characteristic of the genus Rhizopus (Ehrenberg, 1820). On WA, the growth was thin with almost transparent mycelial growth.

Figure 3; Fallen wood rot samples collected in UPLB Baker Hall. A: from front, B: from back, C: from left side and D: from right side.

Measurement of colony diameter was conducted only a day after incubation because the fungal isolates completely proliferated the entire plate only after 2-3 days. This suggested that the isolated fungi were robust and fast growers. Largest colony growth was observed in MEA, where WRF1 (5.00 ± 0.19 cm), WRF8 (5.60 ± 0.43 cm) and WRF5 (5.15 ± 0.25 cm) top the list. Table 2 summarizes the colony size of the fungal isolates in the three different culture media.

Table 2; Average colony growth rate (cm/day) of the wood rot fungi isolates.

Isolate	WA	MEA	PDA
WRF1	3.78 ± 0.39^{a}	5.00 ± 0.19^{abc}	3.95 ± 0.17^{c}
WRF3	3.45 ± 0.05^{ab}	4.93 ± 0.08^{bc}	4.98 ± 0.08^{ab}
WRF5	3.35 ± 0.15^{ab}	5.15 ± 0.25^{ab}	4.65 ± 0.09^{b}
WRF8	1.95 ± 0.09^{c}	5.60 ± 0.43^{a}	5.15 ± 0.23^{a}

Note: WA: water agar, MEA: malt extract agar, PDA: potato dextrose agar. Means within the same column with the same letter superscript are not significantly different at $P<0.0001$ (Tukey's Test)

In terms of colony characteristics, Gams and Bissett (2002) stressed that only some species of *Trichoderma* impart yellow pigmentation on MEA and in PDA. In WA, only thin mycelial growth was observed which was essentially hyaline aerial mycelia, initially submerged but eventually become variably matted. Colony characteristics exhibited by most isolates suggest the genus *Trichoderma* (WRF1, 3, 5) was most predominant and with only one isolate of the genus *Rhizopus* (WRF8) was identified.

Morphological characteristics affirmed that *Trichoderma reesei* in the *Trichoderma* wood rot complex.

Trichoderma spp. can be readily identified by a distinctive set of colony and morphological characteristics. This includes rapid mycelial growth, green or white conidial pigments, and a repetitively branched, but otherwise poorly defined conidiophore structure (Gams and Bissett, 2002).

Although at the species level, there is difficulty in delimiting the isolates because of the very narrow morphological variation and simplified morphology of the genus. Figure 6 summarizes the associated structures and colony growth of the *Trichoderma* WRF5 isolate. As previously stated, WRF5 grows fast in PDA and MEA and imparts yellow pigment on the media. Green powdery fungal growth was observed in the fallen rotten logs after a seven-day incubation in a moist chamber.

Microscopically, the isolate has a branched but poorly defined conidiophore structure as described by non-extensive branching. This is in addition to Ochoa's (1978) observation on his isolate of *Trichoderma*, which he described with hyaline mycelia with smooth-walled, branched, septate conidiophores. These conidiophores were slightly enlarged at the apex, forming the phialides which are mostly inflated. Conidia were light green, ellipsoid, not catenulated, spruce singly and successively. Aggregates of conidia in the culture plate formed distinct conidial heads.

Aside from cultural characterization, morphometric measurements were also conducted to determine the dimensions of the conidia. Average length of 3.19 ± 0.33 µm and width of 2.73 ± 0.36 µm of 20 random conidia were recorded. The morphological characteristics observed in this study conform with the description published by Simmons (1977) when *T. reesei* was first reported, as shown in Figure 5. Thus, the WRF5 isolate was classified as *T. reesei* (Simmons, 1977; Medve et al., 1998; Gams and Bissett, 2002; Martinez et al., 2008).

***In vitro* wood degradation test of T. reesei confirmed its role in wood rot.**

White fungal growth which turned dark green color was formed on the surface of wood blocks as early as one week after inoculation. This observation indicates that the wood (*Shorea palosapis*) can act as a suitable host of the fungi. Fungal growth was also sustained after 14 weeks of incubation, indicating that the wood was a suitable substrate for fungal growth. Figure 6 shows the observed fungal structures on the inoculated wood block under dissecting microscope. The observed structures were compared and re-associated with the previous observations on the rotten wood sample. Relative to the control setup which losses only approximately 1.60% of its weight after 14 weeks, the average percent weight losses of three blocks inoculated with *Trichoderma* sp. was found out to be 8.81 ± 0.79% (8 weeks) and 10.53 ± 0.88% (14 weeks). This signifies that indeed, there is degradation of the polymeric components of the inoculated wood, although the process is very slow. These polymers were reduced to the simpler forms such as glucose that is utilized by the fungus to sustain its growth.

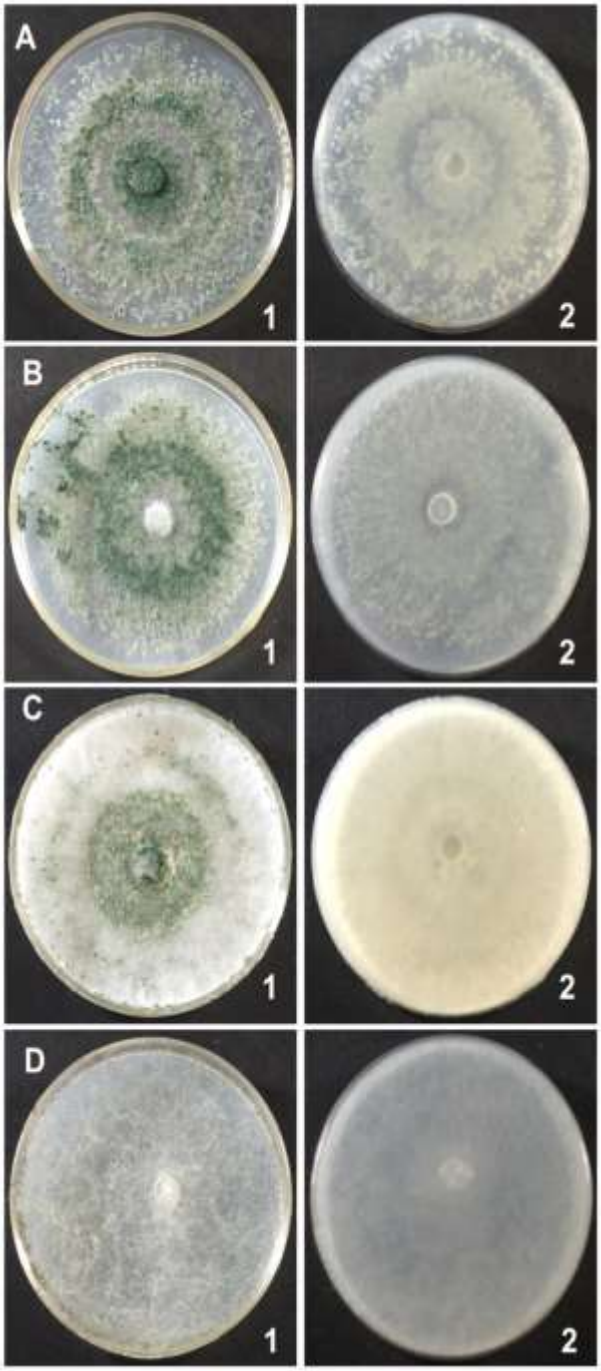

Figure 4; Colonies in PDA of isolated wood rot fungus in UPLB Baker Hall. Panel A: WRF1, Panel B: WRF3, Panel C: WRF5, Panel D: WRF8, Plate 1: Top view and Plate 2: Bottom view.

The results gathered in this experiment collectively suggest the involvement and contribution of Trichoderma reesei in wood deterioration of the canopies in Baker Hall. Trichoderma reesei was reported as a prolific producer of cellulolytic enzymes and is widely used in fermentation and biofuel industries (Henrissat et al., 1985; Tomme et al., 1988; Ilmen et al., 1997; Cumagun et al., 2009; Shrestha et al., 2009). Just recently, the genome of the fungus was sequenced paving the way for genetic engineering of enzymes involved in biomass degradation and biofuel production (Martinez et al., 2008).

Table 3; Average percent weight loss of the wood blocks inoculated with isolate WRF5 (*Trichoderma reesei*).

Sample	8 wks	14 wks
Block 1	9.26%	11.76%
Block 2	7.69%	9.89%
Block 3	9.47%	9.93%
Average ± s.d.	**8.81±0.79%**	**10.53±0.88%**
Control	0.80%	1.60%

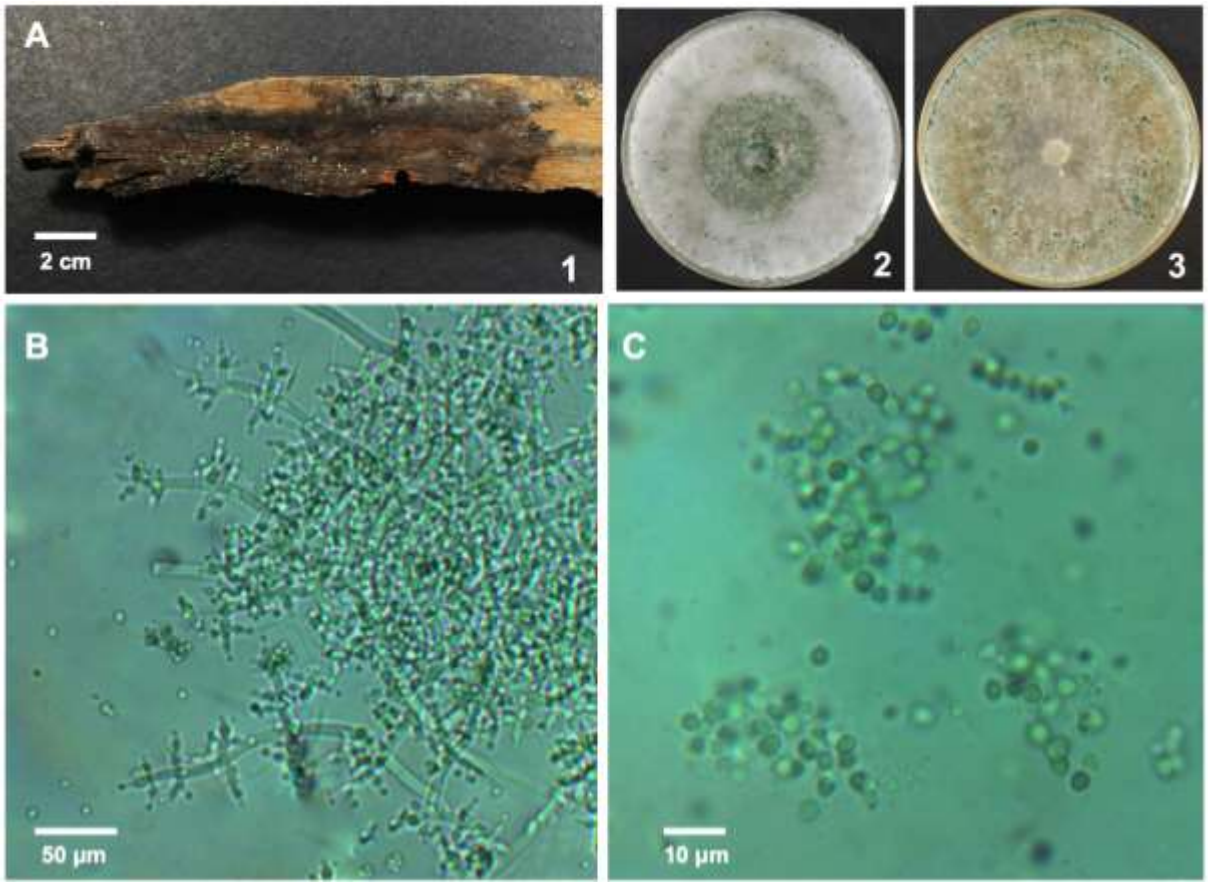

Figure 5; Fungal structures observed in isolate WRF5 which was identified as *Trichoderma reesei* based on morphological characteristics and measurements. Panel A-1: fungal outgrowths of *T. reesei* on fallen rotten wood, Panel A-2: colony in PDA, Panel A-3: colony in MEA, B. conidia borne on conidiophore (400X) and C: conidia (1000X).

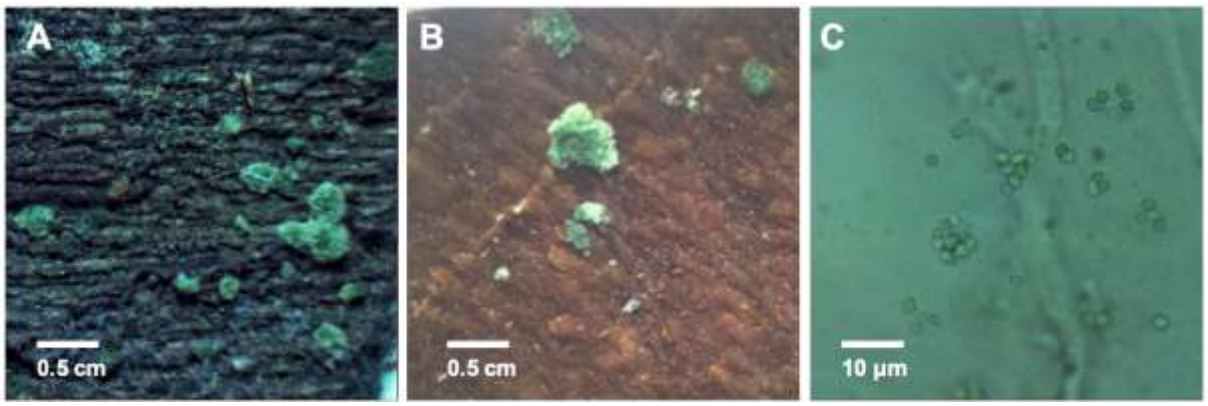

Figure 6; Fungal structures associated with isolate WRF5 (*Trichoderma reesei*). A: Fruiting bodies in the rotten wood block sample observed after seven days of incubation in a moist chamber (40X), B: Fruiting bodies in inoculated wood block observed after two weeks of incubation and C: Conidia of *T. reesei* from the inoculated wood block.

CONCLUSION

Fungi associated with the degradation of Baker Hall wooden elements were identified and their degrading ability was evaluated. Affected wood components show brown color, soft and fibrous textured rot accompanied by the presence of white and green fungal growth. Colonies of the isolates were characterized on PDA, MEA and WA. In PDA, the observed growth was generally raised, light green center with green compacted or flat mycelial tuft and white mycelial growth near the margin. In MEA, colonies were raised with green mycelia at the center covering the whole plate, almost similar to growth observed in PDA. Yellow pigmentation on the agar was imparted by isolate WRF5 only. On WA, colonies were flat with transparent rhizoidal growth only. Fungal isolates were fast-growing in MEA and PDA with colony diameter of 5.60 ± 0.43 cm (WRF8) and 5.15 ± 0.25 cm (WRF5) after a day of incubation. Moreover, morphometric measurement revealed that the average length and width of 20 random conidia of WRF5 were 3.19 ± 0.33 µm and 2.73 ± 0.36 µm, respectively. Based on the colony size and characteristics, related literature and on-site diagnosis, the fungal isolates identified were the mold fungi *Trichoderma viride* (WRF1), *T. crassum* (WRF3), *Trichoderma reesei* (WRF5), *Rhizopus* sp. (WRF8) and the brown rot fungus *Coniophora* sp.Lastly, inoculation on wood blocks of *Shorea palosapis* was performed and the associated fungal growth was observed after 8 and 14 weeks of incubation. Profuse compatible growth of the fungi was observed as early as one week after inoculation. Average percent weight loss was recorded at 8.81 ± 0.79% (8 wks) and 10.53 ± 0.88% (14 wks), which indicates slow wood degradation. The results collectively imply the involvement and contribution of *Trichoderma* spp. in the degradation of the canopies in historic Baker Hall.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

MPSR did the conceptualization, experiment design, executed and coordinated the activities as well as wrote the manuscript and edited until publication. EPP and SFMD helped in the execution of activities and manuscript preparation and editing until publication. CSF contributed to the conceptualization, provision of materials and preparation of the initial draft of the manuscript.

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