

Occurrence of filamentous fungi on *Dendrocalamus giganteus* in Brazil

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Abstract

Bamboo has many economical and environmental advantages compared with other materials commonly employed in construction. However, bamboo is handicapped by the low natural durability of the most of species. According to optimal environmental conditions, several insects or fungi decay bamboo.

The aim of this research was to identify taxonomically some filamentous fungi that decay bamboo in contact with the soil. Fungi were collected from samples of bamboo strips expose to outdoor conditions. Isolated fungi were taxonomically characterized based on morphological and genetic (Amplified Ribosomal DNA Restriction Analysis-ARDRA) approaches.

Ten isolates of filamentous fungi were obtained. Data derived from ARDRA analyses showed the presence of seven different taxonomic groups (ribotypes). Based on microscopic and macroscopic analysis, fungi were identified as belonging to cellulolytic genera: *Arthrinium*, *Fusarium*, *Acremonium*-like and *Trichoderma*, and an unidentified isolate. As there was no fungal mycelial growth of green in samples of bamboo, *Trichoderma* sp. may have been originated from the proper soil. In addition, the fungus that was evaluated separately showed morphological characteristics similar to those of basidiomycete (Basidiomycota).

Introduction

In global terms, 40% of energy consumption and carbon emission in the world are caused by construction (Ferraz 2008). This situation is exacerbated by the use of native timber for

building. According to Kageyama (1987), the deforestation of tropical rainforest may cause the extinction of entire species. The market preference by certain tropical woods because of its high quality, provoke its intensive use and became a serious problem, especially at Sao Paulo State, Brazil.

The solution to this problem involves the use of materials less harmful to the environment than that conventional ones. The possibility of applying bamboo, therefore, appears as an alternative to the tropical wood.

However, bamboo applications are hampered by the low natural durability of the most of specie. Decay caused by physical, chemical and biological agents associate bamboo as a low quality material, creating the false idea that bamboo should be employed only in the scarcity of most appropriate materials.

Fungi were considered plants for a long time. Unlike plants, fungi are heterotroph and do not have chlorophyll or other photosynthetic pigment. Their cell walls are composed by chitin, cellulose does not, unless some aquatic fungi.

Fungi can decompose dead matter (saprotrophic) or obtain its nutrients from living organisms (parasitic), preferring simple carbohydrates, but may also use more complex sources, as starch and cellulose (Burton & Engelkirk 2005). Basidiomycetes fungi are the most responsible by decay materials composed by lignin and cellulose. This group is represented by mushrooms, puffballs and bracket fungi, most of them known for its economic importance, provoking plant diseases, or acting as decomposers of organic matter and for its culinary potential. However, representatives of others fungi groups, such as ascomycetes are able to colonize and degrade lignocelulosic material (Sette et al. 2008).

According to Highley (1999), “fungus damage to wood may be concerned to three general causes: lack of suitable protective measures when wood storing, improper seasoning, storing, or handling of the raw material produced from the log and failure to take ordinary simple precautions in using the final product”.

From the 1980's, many studies on the degradation of wood by fungi were performed. Auer et al. (1988) associated the monoculture of eucalyptus and the high incidence of fungi. Wood has great potential as building material since it is well applied to buildings and since they were well designed, constructed properly and adequately maintained. However, any of

these aspects is often overlooked at the construction, allowing the attack of the decay agents, such insects and fungi (Nunes et al. 2000).

The objective of this research was to identify taxonomically some fungi that decay bamboo in contact to the soil. In a next step, intends to inoculate these fungi on bamboo, seeking to evaluate the effectiveness of some treatments applied to bamboo strips.

Materials and Methods

Figure 1 shows the flowchart of the steps undertaken during the development of this research.

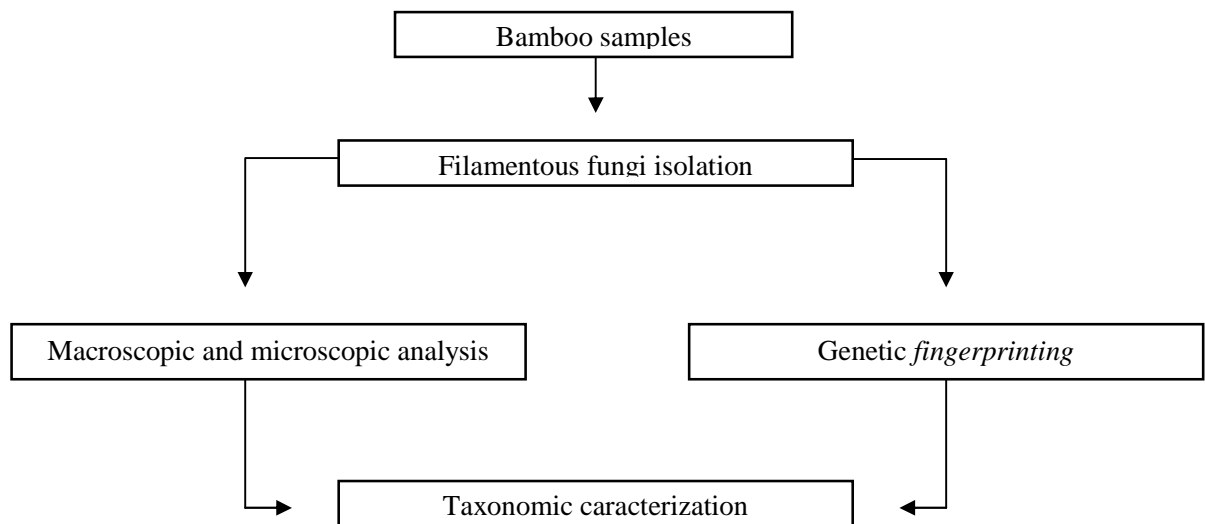


Figure 1 - Flowchart for the implementation of isolation and identification of fungi associated with bamboo.

Strips of 15 cm x 3 cm x 3 cm were obtained from a 5 years old culm of giant bamboo (*Dendrocalamus giganteus* Munro) Strips were exposed to an oxisol type, simulating the decay by wet soil fungi, allowing the colonization of several species (Figure 2).



Figure 2 – Aspect of bamboo strip after 15 days of exposition.

After 15 days of exposure, strips were numbered and delivered to Division of Microbial Resources (CPQBA/UNICAMP) for filamentous fungi isolating and identifying.

A visual inspection of the bamboo strips indicated the development of several fungi (Figure 3), which were readily separated by the morphological characteristics of the colonies. Bamboo strips were washed with sterile distilled water to remove the soil and to isolate only the fungi associated with bamboo. Filamentous fungi were plated by swab technique on culture media MA2 (Malt Extract Agar 2%) and SDA (Sabouraud Dextrose Agar) added 300 mg/L rifampicin, antibiotic to prevent bacteria proliferation. The plates were incubated at laboratory temperature (28 ± 1 °C) for 15 days. Isolation of colonies was conducted daily and pure cultures were obtained after serial transfers on MA2 medium (Figure 4).



Figure 3 – Fungi spores (dark spots) and mycelia (white areas) infesting bamboo.



Figure 4 – Culture of bamboo-derived fungus grown in Petri dish.

The colonies were observed by stereoscope to the fungi identification. Microscope slides were prepared by scrubbing technique, stained with lactophenol cotton blue and visualized in optical microscope. These observations, using the morphological criteria determined by the literature, allowed the preliminary identification of some genera. The identification of species requires molecular techniques (sequencing and phylogenetic analyses) and additional macro and microscopic analyses.

Isolates were subjected to ARDRA analyses (Amplified Ribosomal DNA Restriction Analysis) to identify possible different taxonomic groups. Filamentous fungi were cultured on MA2 medium and after culture growth, genomic DNA extraction was performed according to Raeder & Broda (1985). The 28S rRNA D1/D2 region of the filamentous fungi were amplified from genomic DNA by Polymerase Chain Reaction (PCR) using the following set of primers, NL-1m (5' GCA TAT CAA TAA GCG GAG GAA AAG 3') and NL-4m (5' GGT CCG TGT TTC AAG ACG 3'). PCRs were performed according to Sette et al. (2006). PCR products were digested using the restriction enzymes *MspI*, *HhaI*, *HaeIII* and *AluI* (GE Healthcare). Restriction reactions were carried out in 2h at 37 °C and the electrophoresis were performed on a 2.5% agarose gel, with a 100-bp DNA ladder, for 2.5h at 230 V.

In addition to the filamentous fungi that have developed in bamboo, it was obtained a fungus fruit body, probably a basidiomycete, from one sample of decayed bamboo.

Results and Discussion

From two bamboo samples, ten isolates of filamentous fungi were identified, based on microscopic and macroscopic analysis, as belonging to the genera: *Arthrinium* (F1, F2, F4, F8, F9 e F10), *Fusarium* (F3), *Acremonium*-like (F5) and *Trichoderma* (F6), and an unidentified isolate (F7) (Table 1). In addition, the fungus that was evaluated separately from a decayed bamboo (F11) showed morphological characteristics similar to those of basidiomycete (Basidiomycota), a well known lignocellulolytic degraded group of fungi.

Table 1 - Data from the morphological characterization and genetic fingerprinting.

Isolates	<i>HaeIII</i>	<i>MspI</i>	<i>HhaI</i>	<i>AluI</i>	Ribotypes	Morphologic id.
F1	A	A	A	A	1	<i>Arthrinium</i> sp.
F2	A	A	A	A	1	<i>Arthrinium</i> sp.
F4	A	B	A	A	1A	<i>Arthrinium</i> sp.
F8	A	A	A	A	1	<i>Arthrinium</i> sp.
F9	A	B	A	A	1A	<i>Arthrinium</i> sp.
F10	A	A	A	A	1	<i>Arthrinium</i> sp.
F3	B	C	B	B	2	<i>Fusarium</i> sp.
F5	A	B	C	C	3	<i>Acremonium</i> -like
F6	C	D	D	C	4	<i>Trichoderma</i> sp
F7	D	E	E	D	5	NI*
F11	A	F	F	C	6	NI*

NI * Non identified.

According to Morakotkam et al. (2007), *Arthrinium* (Xylariales) are a dominant genus in bamboos. Representatives of this genus and its telemorph (*Apiospora*) have been reported as fungi associated with bamboo from New Zealand and Japan (Morakotkam et al. 2007). The genus *Fusarium* (Hypocreales) were also reported as fungal associated with bamboo plants (Hino & Katumoto 1961; Morakotkam et al. 2007). *Arthrinium* and *Fusarium* are soil-inhabiting fungi that could be found in decomposing plant material. Both are cellulolytic,

but this activity for *Arthrinium* is rarely reported. *Fusarium* and its anamorph *Giberella* have been isolated from many plants and cause some plant diseases (Rubini et al. 2005).

There are no data in the consulted literature concerning *Acremonium* (anamorphic fungi) and *Trichoderma* (Hypocreales) derived from bamboo samples. As there was no fungal mycelial growth of green in samples of bamboo in the present study, *Trichoderma* sp. (F6) may have been obtained from the proper soil where the bamboo was removed. It is worth to mention that representatives of *Trichoderma* and *Acremonium* are very common in soil and are also able to produce cellulolytic enzymes, which are responsible for cellulose degradation (Nakari-Setälä & Petillä 1995; Stenberg 2004; Ikeda et al. 2007).

Some of isolated fungi showed morphological features very similar and to verify the genetic diversity (polymorphism) of them ARDRA analyses were carried out (Figure 5 and Figure 6). The band pattern (ribotype) generated by enzymatic digestion allowed the differentiation of taxonomic groups previously obtained by conventional taxonomy. Seven different ribotypes were obtained: ribotype 1, 1A, 2, 3, 4, 5 and 6 (Table 1).

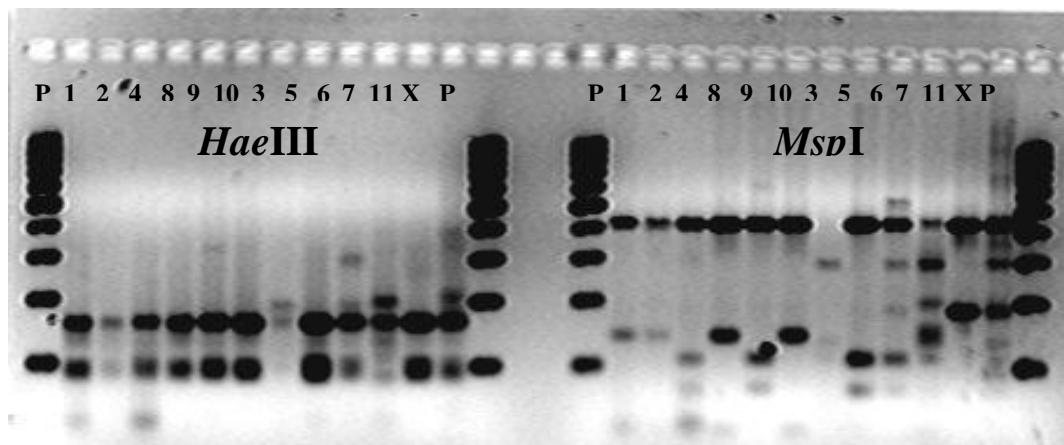


Figure 5 – Restriction profile from the eleven isolated after digestion with the *HaeIII* and *MspI* enzymes. The numbering at the top of the figure represents the fungi order of application on the agarose gel. P = Standard molecular weight (1kb). X = Sample to be disregarded because it is not part of this project.

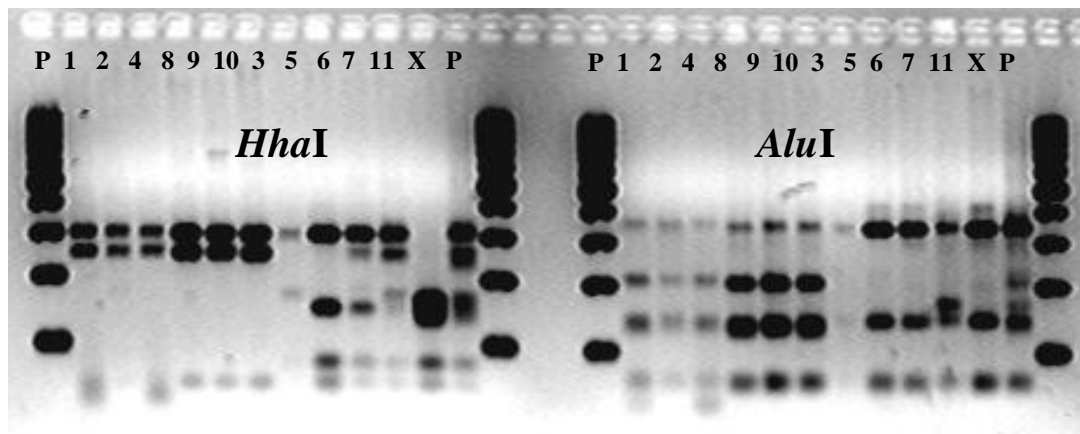


Figure 6 – Restriction profile from the eleven isolates after digestion with the *HhaI* and *AluI* enzymes. The numbering at the top of the figure represents the fungi order of application on the agarose gel. P = Standard molecular weight (1kb). X = Sample to be disregarded because it is not part of this project.

Ribotypes 1 and 1A showed little difference in the restriction profile when *MspI* enzyme was used. As both isolates showed morphological (macroscopical and microscopical) characteristics similar to the genus *Arthrinium*, the polymorphism may not be representative or may indicate strains of different species.

On the other isolates, the results of ARDRA corroborated the morphological analysis, since the fungi showed different restriction profiles and were classified morphologically as belonging to different genera. The isolated fungi unidentified by conventional taxonomy (F7 and F11) showed morphological characteristics and restriction profile different from the others, suggesting that it should belong to different filamentous fungi genera.

Aiming at a more accurate identification of different ribotypes obtained in this work, as well as the identification of ribotypes F7 and F11 (not identified by conventional taxonomy), further studies of sequencing and comparative analysis should be performed.

Conclusions

Although a definitive taxonomic assignment of the fungi isolated and characterized in this study was not always possible, these data present an emerging view of filamentous fungi from Brazilian bamboo samples, since, to our knowledge, there were no previous reports on

fungi isolated from bamboo in Brazil. Based on the literature, the genera *Arthrinium* and *Fusarium* have been reported as fungi associated with bamboo in other countries. However, it is important to highlight that it is the first report concerning *Acremonium* from bamboo samples.

The occurrence of cellulolytic fungi in bamboo was expected, since these fungi are able to use the bamboo cellulose as carbon source. The filamentous fungi isolated in the present study will be deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI) for further research on effectiveness of some treatments applied to bamboo strips against these cellulolytic filamentous fungi.

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