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Evaluation of Competitive Saprophytic Potentials of Microbial Isolates from Some Woods exposed to Indoor and Outdoor Atmospheric Conditions in Uyo, Akwa-Ibom State

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Abstract
Wood deterioration is one of the major effects of heavy economic losses. The rate of deterioration of woods stored or piled for sales are widely studied and well known, the agent responsible for decay of woods in suspended or common roofing conditions has not been extensively studied. This study evaluated the microbial-deteriorating agents that may plausibly influence the strength of woods commonly sold in Uyo timber markets and the effects on the wood when used in roofing. Using standard aerobic culture techniques and analytical procedures, the microbial bio-deterioration agents of woods and their saprophytic potentials were investigated. The result revealed that microorganisms are commonly deposited on Gmelina, Obeche and Iroko wood samples exposed in suspended atmospheric conditions. The microbial isolates were comprised of six fungal and seven bacterial species. The fungal community and their percentage occurrences were as follows: Monillia (30.0%), *Aspergillus fumigates* (16.7%), *Aspergillus niger* (73.3%), *Rhizopus stolonifera* (50.0%), *Candida utilis* (20.0%) and Mucor (56.7%) species, while the bacterial community comprised of *Staphylococcus albus* (20.0%), *Bacillus subtilis* (80.0%), Streptococcus (3.33%), *Staphylococcus aureus* (10.0%), Micrococcus (3.33%), *Bacillus cereus* (80.0%) and Streptomyces (46.7%) species. The microbial isolates exhibited a weak competitive saprophytic potential under their conditions of exposure. The research findings have shown that though the associated micro-organisms possessed a weak enzymatic potential, the fungal components which exhibited a better hydrolytic enzymes potential may grow and produce pigments that may reduce the quality of woods. It is therefore, recommended that proper treatment with wood preservatives be employed to enhance the usage, life and quality of woods used in suspended platforms.

Keywords: Competitive, Saprophytic potentials, Isolates, Atmospheric conditions

Introduction

Wood is often considered as an almost ideal construction material, but a lot of organisms, specifically certain bacteria, fungi, insects, crustacea and mollusc see it as food (Duarte *et al*., 2011). These organisms make wood biodegradable and are therefore crucial to the breakdown of woody materials on the forest floor or in water. The general concept of wood decay has usually involved the actions of higher form of microorganisms especially the fungi such as Basidiomycetes and Ascomycetes. It is only recently that the lower groups; bacteria and actinomycetes have been considered as playing a part in the decay of woods. (Bugg *et al*., *2010*). These micro-organisms are an extremely wide spread and successful group. They are capable of colonizing under conditions such as water logging and low oxygen content that would be unfavorable to most fungi (Bjordal **et** *al*., 1999).

Extensive wood damages are however, usually noticed on woods colonized by the soft rot fungi, wood rotting Basidiomycetes, white rot fungi and brown rot fungi. Bacteria are also known to cause damage in wood with high moisture content, either fresh from the tree, water sprinkled for long term storage prior to sawing or submerged in lakes or wet soil. Bio-deterioration is dependent upon many factors including temperature, microbial population, degree of acclimatization, accessibility of nutrient, cellular transport properties and chemical portioning of growth medium. The minimum, maximum and optimum temperature required for growth varies with different decay fungi. Mohebby (2003) reported that wood decay fungi require wood moisture content in excess fiber saturation point to propagate, fungal growth below fiber saturation point (absence of lumen water) is greatly retarded and that below 20% wood moisture content; their development is completely inhabited. Decay fungi require the free water (lumen water) whereas, sap stain can occur even with bonded water. Another important aspect that may

significantly affect the degradation rate of wood is the kind of wood (softwood or hardwood). Hardwood and softwood differ in several aspects, like fiber dimensions, chemical component composition, lignin and cellulose content. The hardwood presents a vessel element and lignin with both guaiacyl and syringyl units. Softwood does not contain vessel element, the lignin being composed mostly of essentially only guaiacyl units (Saka, 1991). Hard wood degrades faster than softwood (Blanchette, 2000).

Wood decay depends on so many environmental factors which vary from place to place. The rate of deterioration of woods stored or piled for sales are widely studied and well known, however, the agent responsible for decay of woods in suspended or common roofing conditions has neither been extensively studied nor its effect on the strength of wood over time investigated. This study therefore, was designed to evaluate the microbial-deteriorating agents and their saprophytic potentials that may plausibly influence the strength of woods commonly sold in Uyo timber markets and their effects on the wood when used in roofing.

Materials and Methods

Collection of wood samples

The hardwood samples namely; *Triplochiton sclerozylon* (Obeche), *Gmelina arborea Linn* (Gmelina) and *Milicia excelsa* (Iroko) were obtained from commercial piles at the Uyo Timber Market in Akwa-Ibom State which lies on the latitude 5° 03' 4.57" N and longitude 7° 56' 0.60" E. Planks of the selected woods were carefully and separately sawn and the pieces transferred into sterile polythene bags. The samples were then transported to the experimental sites at the University of Uyo Permanent Site.

Laboratory analysis of wood samples

The wood samples were sawn into sizes of 1.25cm and 15cm width by length respectively. The sized wood samples were hung 7cm apart on a metal clamp using strings and exposed outdoor in the field and indoor at the University of Uyo Permanent Site. Bi weekly analysis of the microbiological properties and tensile strength of the exposed wood samples were determined before exposure and during deterioration for 18weeks using the destructive approach as described by Vainio-Kaila *et al.* (2010). After every two weeks of exposure, representative samples of each of the exposed wood samples were removed from the line, sized into pellets using sterilized knife and then crushed into powdery form using sterile laboratory mortar and pestle. Exactly 2g of the milled sample were suspended in 18ml of sterile distilled water. A ten-fold serial dilution of the supernatant solution derived from each milled sample of the exposed wood sample was carried out. The dilutions of 10^{-3} and 10^{-4} were subjectively chosen for the enumeration and isolation of heterotrophic bacteria and fungi. Analyses of the extracellular enzymatic activities of the microbial isolates were performed using media that differentiate organism able to produce extracellular cellulases, amylases, lipases and proteases. For bacterial growth, all media were adjusted to pH7, while test fungal isolates were initially grown on streptomycin supplemented Sabourand Dextrose Agar (SDA) at pH5.7. Antibiotics were added to the molten medium (48ºC) just prior to pouring plates to provide a final concentration in the medium of 100 and 50ugml-1 respectively. The nutrient agar (NA) plates were incubated at 37ºC for 24 hours, while the SDA plates were incubated at room temperature (28±2ºC) for 5days. Pure culture of the microorganisms were obtained by repeated sub-culturing and used as source of inocula for the enzyme assay. The strength of the wood exposed indoor and outdoor was determined using Young Modulus formula (Serway and Faughn, 2003). The elastic modulus otherwise called the Young's modulus (Y) of a material determines the stiffness of that material. It is a suitable quantity that relates the stress and strain that best measures the degree of deformation or degradation of a material. Materials with large elastic modulus are expected to be stiffer and could withstand large stress and difficult to be stretched or deformed. A cantilever experiment was conducted to measure the Young modulus of the wood, where the initial strength of the wood was determined and later determined after 3months for a period of 6months' exposure. The wood samples were Iroko, Obeche and Gmelina. The wood samples of 60cm sizes were weighed to determine their weights using a Hanson balance model 928 before the experiment; also the width and thickness were measured with Veneer calipers. The sample was clamped firmly to the edge of a work bench with a length of 85.0cm projecting from the edge of the bench. A mass of 100g was attached to the free end of the projected sample to deform it. The projected sample was caused to vibrate for 30 times and the period of vibrations calculated. The experiment was repeated for varying lengths in steps of 5.0cm of the fresh sample, then after the sample was exposed in indoor and outdoor environment, again after another exposure (Faughn and Serway, 2003).

Results and Discussion

Results

The fungi and bacteria associated with the bio-deterioration of the exposed wood samples were isolated and characterized as presented in Table 1. The attributes of isolates revealed the presence of six and seven fungal and bacterial species respectively on the exposed wood samples. Their prevalence on the wood substrates however varied with the type of wood and exposure condition. *Penicillium* sp, *Aspergillus niger*, *Bacillus subtilis* and *Bacillus cereus* were the most prevalent wood deteriogens in indoor environment, while Mucorsp, *Aspergillus niger, Bacillus cereus* and *Bacillus subtilis* prevailed in woods suspended in the outdoor environment.

Catabolic Potential of Diverse Microbial Deteriogens

The enzymatic activities of the fungal and bacterial isolates are presented in Table 2. The results revealed a weak competitive saprophytic potential of the microbial isolates under their conditions of exposure. The fungal isolates elaborated a wide range of hydrolytic enzymes.

Their potential to degrade the wood samples were generally moderate, although, *Monilia spp.* exhibited the weakest catabolic potential. On the other hand, the bacterial isolates exhibited varied enzymatic potentials. Many of the bacterial isolates failed to elaborate lipase and amylase in test media, but readily produced protease enzyme and only few of them; *Bacillus subtilis*, *Bacillus cereus* and *Streptomyces spp*. elaborated cellulase enzyme.

Discussion

The quality of woods gets deteriorated due to the action of micro-organisms, particularly fungi. The micro-organisms isolated from the exposed wood samples in this study were fungi (*Penicillium citruim*, *Aspergilllus niger*, *Aspergillus fumigates*, *Monilia spp.*, *Rhizopus stolonifer*, *Mucor miehei* and *Candida* utilis) and bacteria (*Streptomyces spp.*, *Staphylococcus aureus*, *Streptococcus spp*, *Staphylococcus albus*, *Micrococcus spp.*, *Bacillus cereus* and *Bacillus subtilis*). Among the isolates obtained from the woods, *Bacillus*, *Streptomyces*, *Candida*, *Aspergillus* and *Penicillium* species have been previously associated with deterioration or decay of woods (Aaron *et al.*, 2011; Blanco 1999). while other mould species may be implicated with wood discolouration through the formation of pigments (Petri *et al*., 2011).

It has been reported that easily available carbon, nitrogen and sulphur favour the growth of micro-organisms. However, products essentially manufactured from the naturally occurring organic materials such as cellulose, hemicellulose, starch and nitrogen can be most susceptible to micro-organisms if the required enzymes are elaborated (Gaur *et al*., 2005). In order to determine the wood deteriorating potentials of the various microbes isolated, their ability to elaborate some enzymes (Cellulase, lipase, Amylase and Protease) were assayed. Combined hydrolytic activities were detected in a number of isolates. The fungal species except *Monilia spp*. had a greater enzyme elaborating potentials than the bacterial isolates. This is believed to have contributed to their ability to strive more on the wood samples. On the other hand, the bacterial isolates exhibited varied enzymatic potentials. Many of the bacterial isolates failed to elaborate lipase and amylase in test media. but readily produced protease enzyme and only few of them, namely *Bacillus subtilis*, *Bacillus cereus* and *Streptomyces spp.* elaborated cellulase enzyme. The cellulolytic activity of *Bacillus* and *Streptomyces* species have previously been reported by Mandels *et al.* (1976).

Cellulose constitutes the most common polysaccharide constituent of plant woods (Hulme and Shields, 1975). Cellulosic material on its own is prone to microbial degradation, but very resistant when in combination with the recalcitrant lignin component as found in woods (Antai and Crawford, 1983). The relative ability of micro-organisms to degrade the lignin-cellulose complex could be the main rate limiting factor in wood deterioration. Essien and Eduok (2001) and Obuekwe and Okungbowa (1986) have reported that enhanced degradation of cellulose can be achieved through the production of both Beta (1-4) glucanase (C1) and carboxymethyl cellulase (Cx). The advantage of the production of the two sets of cellulases rests on the joint catabolic mechanisms of both exo and endo-enzymes. The C1 enzyme is reported to initiate the degradation of native cellulose by dis-aggregating the β 1-4 glucan, while Cx enzyme is associated with the hydrolysis of the cellobiose into soluble sugar. The results have also shown that the slow rate of decomposition of woods suspended in air may also be attributed to the relatively poor enzymatic potentials of the microbial colonizers. The organisms lack an "overcoming" enzymatic suite necessary for effective degradation of woods in suspended atmospheric conditions.

Conclusion

The results of this study have revealed that woods kept off soil would last longer when used in roofing purposes. This is based on the fact that that wood exposed in suspended atmospheric conditions is a poor substratum for microbial growth and subsequent degradation. The research findings have shown that although the associated micro-organisms possessed a weak competitive enzymatic potential, the fungal component of the deteriogens which exhibited a better hydrolytic enzymes potential may grow and produce pigments that may reduce the quality of the woods. It is therefore, recommended that proper treatment with wood preservatives be employed to enhance the useful life and quality of woods used in suspended platforms.

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Table 1: Occurrence of Microorganisms Isolated from the Exposed Wood Samples.

Isolates	Obeche (n = 10)	Gmelina (n = 10)	Iroko (n = 10)	Frequency of Occurrence	% Occurrence Rate
			Indoor		
Mucor sp	7	7	3	17	56.7
Aspergillus niger	8	8	6	22	73.3
Penicillium sp.	8	7	5	20	66.7
Aspergillus fumigatus	3	2	0	5	16.7
Monilia sp.	4	3	2	9	30.0
Candida utilis	1	3	2	6	20.0
Rhizopus stolonifer	7	5	3	15	50.0
Staphylococcus albus	3	_	4	7	23.0
Bacillus subtilis	8	8	6	22	73.3
Staphylococcus aureus	3	3	1	7	23.0
Micrococcus sp.	3	3	_	6	20.0
Bacillus cereus	8	8	6	22	73.3
Streptomyces sp	4	4	6	14	46.7
			Outdoor		
Mucor sp.	10	6	4	20	66.7
Aspergillus niger	9	8	7	24	80.0
Penicillium sp.	6	6	4	16	53.3
Aspergillus fumigatus	2	4	_	6	20.0
Monilia sp.	2	2	_	4	13.3
Candida utilis	2	2	1	5	16.7
Rhizopus stolonifer	7	7	3	17	56.7
Staphylococcus albus	4	2	2	8	20.0
Bacillus subtilis	9	9	6	24	80.0
Streptococcus sp.	1	-	-	1	3.33
Staphylococcus aureus	-	-	3	3	10.0
Micrococcus sp.	1	-	-_	1	3.33
Bacillus cereus	9	9	6	24	80.0
Streptomyces sp	4	4	6	14	46.7

Table 2: Enzymatic potential of the fungal and bacterial isolates

Isolate	Cellulase	Lipase	Amylase	Protease
Enzymatic potential of the fungal isolate				
Mucor miehei	++	+	++	++
Aspergillus niger	++	+	++	++
Penicillium citruim	++	++	++	+
Aspergillus fumigatus	++	++	++	++
Monilia sp	+	-	-	++
Candida utilis	+	+	++	-
Rhizopus stolonifer	++	++	++	+
Enzymatic potential of the bacterial isolate				
Staph albus	-	-	-	+
Bacillus subtilis	++	+	++	++
Streptococcus sp	+	-	-	++
Staph aureus	-	-	-	++
Micrococucus sp	+	-	-	+++
Bacillus cereus	++	+	+	+
Streptomyces sp	++	-	+	++

Key: +++ = high activity, ++ = moderate activity, + = low activity and – = no activity