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Studies on the Effect of Some Wood Extracts on Growth and Cellulase Production by Strains of *Bacillus subtilis*

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Abstract: The effect of aqueous extracts of five wood samples: *Khaya grandifoliola*, *Mansonia altissima*, *Brachystegia eurycoma*, *Milicia excelsa* and *Terminalia superba* on some cellulolytic bacterial strains was investigated. The aqueous extracts of the wood samples inhibited the growth and cellulolytic activity of the *Bacillus subtilis* strains to varying degrees. The inhibitory effect of the extracts of *Brachystegia eurycoma* against the bacterial strains was highest when compared to the other wood types.

Key words: Wood extracts, cellulase, inhibitory, *Bacillus subtilis*

INTRODUCTION

Termites are responsible for much of the degradation of wood and other cellulose materials in the Tropics and sub-Tropics (Peralta *et al*., 2004). While some wood are resistant to their attack, others are not (Nakayama *et al*., 2000; Peralta *et al*., 2004). Cellulose form the principal food of wood-eating termites. Termites' gut microflora have been reported to play vital role in carbon nutrition and energy derived from digestion of cellulose (Breznak and Brune, 1994). The lower termites possess intestinal protozoa which help them in the digestion of wood, while the higher termite which lack intestinal protozoa possess bacteria in their hindguts.

The hindgut of termites are colonized by diverse group of bacteria such as *Bacillus*, *Micrococcus*, *Streptococcus*, *Bacteroides*, *Streptomyces*, *Staphylococcus* and various Enterobacteriaceae (Amund *et al*., 1986; Femi-Ola *et al*., 2001).

Cellulose hydrolysis is accomplished with the aid of cellulase enzyme complex which is made up of three classes of enzymes namely exoglucanase, endoglucanase and β glucosidase (Beguin, 1990). The cellulolytic organisms in the hindgut of termites are known to produce extracellular cellulases necessary for the hydrolysis of cellulose. Certain extractives from some woods have been reported to have termicidal properties (Duryea *et al*., 1999; Femi-Ola *et al*., 2007; Nakayama *et al*., 2000; Neya *et al*., 2004). However, there is paucity of information on the effect of these extractives on the microflora in the hindgut of the termites. Thus, this study investigated the effect of aqueous extracts of some woods on the growth and cellulase production in some strains of cellulolytic *Bacillus subtilis*.

MATERIALS AND METHODS

Culture: *Bacillus subtilis* (NCIB 3610) was obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife. The study was conducted during the wet season. *Bacillus subtilis* (BS5) was isolated from the hindgut of wood-eating termites *Amitermes evuncifer* (Silvestri) (Femi-Ola *et al*., 2001). Bacterial suspension was prepared in normal saline from 24 h-old slant culture. The suspension was diluted to give an extinction of 0.20. The total bacterial count in the suspension was approximately 54×10^9 organisms mL^{-1}.

Wood materials: Wood chips of *Milicia excelsa* (Welw) C.C. Berg, *Mansonia altissima* Chev, *Khaya grandifoliola* C.Dc., *Brachystegia eurycoma* Harms and *Terminalia superba* Engl and Diels were collected from Bashiri Sawmill in Ado-Ekiti, Ekiti-State, Nigeria.

Preparation of extracts: Twenty grams of the wood shaving of each wood sample were suspended in 100 mL of distilled water in 250 mL flask. The mixtures were held for 90 min in a water bath at 80°C; after which they were allowed to stand for 3 days. Each extract was filtered through sterile Millipore filter (0.22 μm) into a sterile flask. Dilution of each extract was prepared in sterile citrate phosphate buffer (pH 6.5) to give a final concentration of 25, 50 and 100 mg mL^{-1}.

***In vitro* test of effect of wood extract:** The effect of wood extracts on cellulase production and cellulolytic bacteria were determined by culturing the test organisms in carboxymethylcellulose medium containing in g L^{-1}:

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K_2HPO_4, 0.2; KH_2PO_4, 0.5; $CaCl_2.2H_2O$, 0.02; $NaNO_3$, 2.0; NaCl, 0.5; $MgSO_4.7H_2O$, 0.2; $MnSO_4.H_2O$, 0.02; $FeSO_4$, 0.02; yeast extract 0.5; Carboxymethylcellulose (CMC High viscosity BDH UK) 10.0. The basal medium was autoclaved at 121°C for 15 mins while CMC was autoclaved separately and added to the basal medium to give a final concentration of 1% (w/v). The medium (25 mL) was supplemented with 1 mL of wood extract and then inoculated with 0.5 mL suspension of the test organisms. Inoculated flasks were incubated at 35°C for 36 h. Growth was determined by measuring the Optical Density (OD) at 470 nm. Uninoculated medium served as control in each concentration, protein content was determined by Lowry *et al.* (1951). Cellulolytic activity was assayed for by the copper arsenomolybdate colour reagent method of Somogyi (1952).

Determination of cellulolytic activity: Cultures were harvested by centrifugation at 5000 rpm for 20 min. Culture supernatants were used for the assay of the extracellular enzyme. To 0.5 mL of 1% CMC in 0.1 M citrate phosphate buffer (pH 6.5) was added 0.5 mL of crude enzyme preparation. The reaction mixture was incubated at 35°C for 1 h in a water bath. The reaction mixture was terminated by heating at 100C° for 10 min, while the cellulase activity was determined by the method of Somogyi (1952). One unit of cellulase activity was expressed as the amount of enzyme required to liberate 1 μg of glucose per minute.

Statistical analysis: Analysis of variance and Schefee pair wise multiple comparison using SPSS version 11.0 software were carried on data obtained from different experimental results.

RESULTS AND DISCUSSION

Addition of the wood extract significantly affected the growth of the *Bacillus* strains ($p<0.05$).The Scheffe pair wise multiple comparison showed that there were significant differences in the growth of the *Bacillus* strains when the wood extracts was absent (control) and when added (Table 1). The inhibitory effect of the aqueous extract of *B. eurycoma* was stronger than the other wood types. The concentration of the wood extracts also had effect on the growth of test organisms; however the differences were not significant ($p>0.05$).

There was significant difference in the amount of protein released by the test organism in the presence and absence of wood extracts ($p<0.05$). There was no significant difference between additions of aqueous extract of *T. superba* and control (Table 2). Statistical analysis also showed that there was a significant difference ($p<0.05$) in protein content on addition of different concentration of wood extracts. Scheffe test showed that there were significant differences ($p<0.05$) between the means of control and wood extracts with concentration 50 and 100 mg mL^{-1}. There was however no significant difference ($p>0.05$) in specific activity of cellulase produced by the different *B. subtilis* strains.

Table 1: Effect of wood extracts on growth of two strains of *Bacillus subtilis*

Wood extract/ concentration (mg mL^{-1})	Growth ($E_{470\ nm}$)	
	B. subtilis (BS5)	*B. subtilis* NCIB3610
Control (no wood extract)	0.37a	0.35a
M. excelsa		
25	0.17c	0.20b
50	0.16c	0.16c
100	0.12c	0.15c
T. superba		
25	0.26b	0.24b
50	0.22b	0.20b
100	0.22b	0.20b
M. altissima		
25	0.19c	0.16c
50	0.09c	0.12c
100	0.06d	0.10c
K. grandifoliola		
25	0.19c	0.19c
50	0.16c	0.16c
100	0.10c	0.09c
B. eurycoma		
25	0.02d	0.04d
50	0.00d	0.00d
100	0.00d	0.00d

Averages within a column followed by the same letter(s) are not significantly different as gauged by Schefee pair wise multiple comparison

Table 2: Effect of wood extracts on cellulolytic activity of *Bacillus subtilis* BS5 isolated from hindgut of *Amitermes evuncifer*

	B. subtilis (BS5)		*B. subtilis* NCIB3610	
Wood extract/ concentration (mg mL^{-1})	Protein content (mg mL^{-1})	Cellulase activity	Protein content (mg mL^{-1})	Cellulase activity
Control (no wood extract)	0.17a	2.47±0.01a	0.15a	3.00±0.01a
M. excelsa				
25	0.11b	0.0b	0.10a	2.00±0.01a
50	0.09b	0.0b	0.06b	1.16±0.05b
100	0.06b	0.0b	0.05b	0.0c
T. superba				
25	0.18a	3.01±0.01a	0.16a	1.75±0.05b
50	0.18a	3.01±0.01a	0.10a	2.25±0.01a
100	0.16a	2.92±0.01a	0.09a	2.27±0.01a
M. altissima				
25	0.06b	0.0b	0.05b	2.31±0.05a
50	0.02c	0.0b	0.02c	1.80±0.05b
100	0.01c	0.0b	0.01c	0.0c
K. grandifoliola				
25	0.09b	2.63±0.01a	0.08b	0.0c
50	0.06b	2.50±0.01a	0.06b	0.0c
100	0.06b	0.0b	0.05b	0.0c
B. eurycoma				
25	0.02c	0.0b	0.02c	0.0c
50	0.02c	0.0b	0.01c	0.0c
100	0.01c	0.0b	0.01c	0.0c

Averages within a column followed by the same letter(s) are not significantly different, as gauged by Schefee pair wise multiple comparison

The study has shown that aqueous extract of the woods inhibited the growth and cellulase production in the strains of *Bacillus subtilis* significantly. Although the growth and the cellulolytic activity of the organisms were affected by the concentration of the wood extracts, the differences were not significant. The celluloytic activity of the two strains of *B. subtilis* was least affected by aqueous extract of *T. superba*. The aqueous extract of *B. eurycoma* most effective, as it inhibited the growth and cellulolytic activity of the *Bacillus* strains at all concentrations employed.

The inhibition might be due to the chemical composition of the woods. Extractives in form of resin, hormones and fatty acids have been reported to account for 3±5% of softwood and 5±3% of heartwoods (Illston *et al*., 1981; Neya *et al*., 2004). These substances and some others have been suggested to contribute to the resistance of some woods to termite infestation (Nakayama *et al*., 2000; Neya *et al*., 2004; Peralta *et al*., 2004).

Amylase and protease inhibitors have been demonstrated in extracts of kolanut (*Cola nitida* and *C. acuminata*) against the protease and amylase of the kolanut weevil (*Sophrorhinus insperatus* Faust) (Adedire and Balogun, 1992; Adedire, 1994). Extractives in the wood that is antimicrobial and inhibitory to cellulase activity may be responsible for the protection of some woods against termite attack.

CONCLUSION

Aqueous extract of some of the woods inhibited growth and cellulase production in *Bacillus subtilis* strains. High concentration of wood extracts (100 mg mL^{-1}) of *Milicia excelsa*, *Mansonia altissima*, *Khaya grandifoliola* and *Brachystegia eurycoma* were effective in inhibiting cellulolytic activity in *B. subtilis* BS5 and *B. subtilis* NCIB3610, respectively. Among the wood species, *B. eurycoma* had the strongest inhibitory effects on the growth and cellulolytic activity of *B. subtilis* BS5, isolated from the hindgut of *A. evuncifer*. Extractives in wood contribute immensely to their protection against termite attack. Aqueous extract of *T. superba* did not have any inhibitory effect on cellulase production by the strains of *B. subtilis*.

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