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Cellulose-degradation potential and cellulase activities of Bacillus and Lysinibacillus species isolated from tropical coastal sediments in Nigeria

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ABSTRACT

Marine microorganisms are known to withstand adverse conditions, which make them potentially useful for bioconversions. In a recent study, cellulolytic species of Bacillus (B. albus, B. thurigiensis) and Lysinibacillus (L. borontolerance, L fulsiformis, L. sphaerocus, L. xylanilyticus) were isolated from the Atlantic Ocean coastal sediments in Nigeria. Considering the potential biotechnological applications of these bacteria, the study was extended to focus on the extent of their cellulose-degradation and cellulase activity capabilities. Obeche (Tryplochiton scleroxylon) wood was exposed to the organisms in Mineral Salts Medium (MSM) for 16 weeks and examined for weight losses before microchemical tests were conducted on the wood sections to detect the component(s) attacked. Whatman Filter Paper was also similarly exposed to the bacteria, but for only 72 h. Supernatants from MSM- and seawater-carboxyl methyl cellulose (CMC) broth were tested for cellulase activities with CMC and were monitored daily for 7 days. Lowry method was used to determine protein concentrations in the medium after which specific cellulase activities were calculated. The organisms caused weight losses in submerged and partially submerged wood, which were not significantly different (20.5-26.1 vs 21.1-25.7%). Microchemical analyses showed that cellulose was degraded by all the organisms while lignin was partially attacked. Filter paper degradation was indicated by weight losses of 3.28-4.18% in 72 h. Cellulase activity values obtained from MSM-CMC broth after 24 h stood at 6.94±0.8-9.79±1.1 U/ml and except for L. borontolerance (P=0.040), they were not significantly different from seawater-CMC broth-derived cellulases (7.75±0.8-9.46±1.2 U/ml). The cellulase activities peaked on the 4th day (27.75-34.11 U/ml). Specific cellulase activity was 2.14-4-03 on the 1st day and 8.21-16.89 U/mg by the 7th day. Thus, the capabilities of species of Bacillus and Lysinibacillus to degrade cellulose and produce substantial cellulases are demonstrated. Purification can enhance the enzymes' activities and make them potentially suitable for biotechnological applications.

Keywords: *Coastal sediment, Bacillus, Lysinibacillus, cellulase activity*

1.0 Introduction

Cellulose is the most abundant carbohydrate in the world, a major component of agricultural wastes and an important renewable energy source [1]. Bacteria and fungi are the major agents of depolymerisation of cellulose to glucose and other soluble sugars via cellulase enzymes and these enzymes have been in commercial use for almost three decades [2]. Cellulase enzymes have been usefully applied in several industries which include textile, pulp and paper and production of fruit juices [3]. Cellulolytic microorganisms -are diverse and can be found in soil, aquatic environment, ruminants and insects especially termites [4]. Basidiomycetes and microfungi are the dominant cellulose-degrading organisms in the terrestrial environment, while bacteria are the main cellulolytic species in the aquatic environment. However, most of the studies on sources of cellulase enzymes especially for industrial use tend to focus more on filamentous fungi than bacteria, especially *Trichoderma, Aspergillus* and *Penicillium* [5].

Cellulase-producing microorganisms in the marine environment including coastlines, have not been given adequate attention by researchers. It is therefore important to extend the search for cellulase-producing bacteria to the marine environment.

The marine environment contains diverse microorganisms that are associated with recycling of nutrients, especially in mangroves, estuaries and coastlines where organic matter including lignocellulosic wastes are deposited [6, 7]. These lignocellulosic wastes are degraded by cellulolytic bacteria hence they can be potential sources of cellulases for industrial use. Microorganisms in the marine environment tolerate extreme conditions which include high salinity, limited nutrients and anoxic conditionswithst. It is therefore not unlikely that cellulase enzymes from marine bacteria can function under stressful conditions; , which is a desirable attractive factor for industrial applications [8, 9]. This is therefore an impetus for searching the marine environment, including coastal sediments for cellulose-degrading bacteria.

In a recent study [10], species of cellulolytic *Bacillus* and *Lysinibacillus* bacteria with cellulolytic indexes ranging from 3.2 to 3.5 were isolated from the Atlantic Ocean coastal sediment in Escravos, Nigeria. This was considered substantial enough to warrant extending the study to focus on the abilities of the isolated *Bacillus* and *Lysinibacillus* species to degrade native cellulose (wood), purified cellulose (filter paper) and their cellulase and specific cellulase activities. The outcome of this study may provide further evidence of the presence of potentially industrially-useful cellulase enzymes from bacteria living in the marine/coastal environment.

2.0 Materials and Methods

2.1 Wood degradation potential of species of Bacillus and Lysinibacillus isolated from coastal sediments.

The cellulolytic *Bacillus* and *Lysinibacillus* species isolated from the coastal sediment in the previous study [10] were used for the degradation tests. Triplicate Obeche wood *(Triplochyton scleroxylon*) blocks measuring 1 x 1 x 1 cm were dried to constant weight at 105 ^{0}C and immersed in 100 ml screw-capped bottles filled with sterile mineral salt medium (MSM). The MSM consisted of: KH_2PO_4, 2.0 g; K_2HPO_4 2.5 g; $MgSO_4.7H_2O$ 0.05 g; NaCl 2.0 g; $MnCl.7H_2O$, 0.0003 g; $ZnCl.7H_2O$, 0.0003 g; yeast extract, 0.5 g; deionized water, 1000 ml. Care was taken to ensure that the blocks were fully immersed without leaving air-space in the bottles. Another set of blocks were was partially immersed leaving parts of the wood blocks exposed to air. Thereafter, the bottles were inoculated with loop-full of the test isolates and left on the laboratory bench at room temperature (30±^{0}C) for 16 weeks. Control blocks were not inoculated. Upon expiration of the incubation period, the blocks were retrieved and dried to constant weight. Thereafter, the weight loses wereses were calculated by subtracting the final weights from the initial weights; the losses were then converted to percentages.

Degradation of `cellulose and lignin in the wood blocks exposed to the isolates was further assessed by microchemical tests on the basis of colour reaction intensities. The procedure described in a previous study [11] was followed. A solution of iodine in potassium iodide and 65% sulphuric acid was used to stain razor blade sections of the wood blocks for the detection of cellulose. A blue-black colour shows the presence of cellulose while absence of the blue-black colour or reductions in intensity to blue/light blue represents complete or partial loss of cellulose, With respect to lignin, detection was by Weisner test which produces bright-red colour with lignin. A decrease in brightness or disappearance of colour shows partial or complete degradation of lignin.

2.2 Filter paper degradation test

Strips of Whatman No 1 Filter paper were dried to constant weight at 105 °C, cooled and immersed in sterile MSM in flasks before inoculation with loopful of the *Bacillus* and *Lysinibacillus* isolates. The flasks were incubated for 72 h at room temperature after which the filter paper strips were removed and dried to constant weight. The weight losses were subsequently calculated as before.

2.3. *Determination of cellulase activity (CMCase)*

The *Bacillus* and *Lysinibacillus* species were each propagated in 1% carboxyl methyl cellulose medium (CMC) which was prepared with MSM in 21 flasks.

The flasks were shaken for 7 days (150 rpm) at room temperature. Triplicate flasks were withdrawn daily and centrifuged at 10000 rpm for 10 mins. The supernatant was the crude enzyme source used for the cellulase activity assay. The above procedure was repeated by substituting MSM with seawater.

The substrate used for the cellulase activity tests was 1% solution of CMC in 0.05 M citrate buffer (pH 4.8). The test was conducted at 50 °C for 60 min using a reaction mixture contains containing 0.5 ml of the supernatant and 1 ml citrate buffer. The reducing sugar (glucose) produced was determined by the dinitrosalicylic acid (DNSA) method [12]. Lowry method was used to determine the protein concentration in the medium [13].

The enzyme activity in U/mL was subsequently calculated using the formula below[14]:

Cellulase activity (U/ml) = µmol glucose released x Volume of reaction mixture (ml)
Volume of enzyme x incubation time (mins)

Specific cellulase activity (U/mg) was calculated with formula: U/ml ÷ protein conc. (mg/ml) [15].

3.0 Results

The results in Table 1 show that all the species of *Bacillus* and *Lysinibacillus* degraded the wood with weight loses close to 25% without marked differences by species. The weight losses were also identical in submerged and partially submerged wood blocks. The microchemical tests showed that cellulose was the main target of all the bacteria while lignin was partially attacked epecially by the two *Bacillus* species (Table 2).

Table 1: Degradation of Obeche (Tryplochiton scleroxylon) wood blocks exposed to the bacteria isolated from coastal sediments for 16 weeks

Isolates	Mean wood weight loss (%)±SD	
	*Completely submerged blocks	*Partially submerged blocks
Bacillus albus	24.4±0.8	23.8±2.2
Bacillus thurigiensis	26.1±2.3	25.7±2.4
Lysinibacillus borontolerance	23.4±1'7	24.0±1.8
Lysinibacillus sphaerocus	23.1±3.2	24.3±2.6
Lysinibacillus fulsiformis	21.2±2.1	22.6±1.8
Lysinibacillus xylanilyticus	20.5±2.3	21.1±2.3

No significant difference in with all isolates (t-test, P>0.05)

Table 2: Microchemical indication of degradation of cellulose and lignin in wood exposed to bacteria isolated from coastal sediments for 16 weeks

Isolates	Colour intensity	
	Cellulose*	Lignin**
Bacillus albus	-	+
Bacillus thurigiensis	-	+
Lysinibacillus borontolerance	-	++
Lysinibacillus sphaerocus	-	++
Lysinibacillus fulsiformis	-	++
Lysinibacillus xylanilyticus	-	++
Control	+++	+++

**Blue-black, +++; blue, ++; light blue, +; -, no colour, ** Bright red, +++; red, ++; dull red, +.*

Table 3 presents the results of the ability of the *Bacillus* and *Lysinibacillus* spp to degrade filter paper. With the exception of *B. paramycoides*, all the weight losses did not markedly differ by species (Table 3). The enzyme activities of the cellulases obtained from bacterial growth in MSM-CMC broth did not also markedly differ by species with the exception of *L. fulsiformis* and *L. borontolerance* that were markedly lower (Table 4).

A similar trend was repeated with enzymes obtained from bacterial growth in seawater-CMC broth; and the activities of the cellulases from both sources were not significantly different except that of *L. borontolerance* (Table 4).

Table 3: Biodegradation of Filter paper exposed to bacteria isolated from coastal sediments

Isolates	Mean weight loss (%)±SD
Bacillus albus	3.28±0.43
Bacillus thurigiensis	4.18±0.56
Lysinibacillus borontolerance	3.48±0.44
Lysinibacillus sphaerocus	3.66±0.55
Lysinibacillus fulsiformis	3.32±0.42
Lysinibacillus xylanilyticus	3.45±0.47

Table 4: CMCase activity of isolates propagated in CMC broth prepared with MSM and seawater after 24 h

Isolates	Mean cellulase activity (U/ml)±SD		Sign. Diff. (*P*)
	MSM	Seawater	
Lysinibacillus xylanilyticus	8.27±1.0	8.50±0.1	0.168
Lysinibacillus borontolerance	7.27±0.8	8.20±1.1	0.040
Lysinibacillus sphaerocus	9.79±1.1	9.46±1.2	0.158
Lysinibacillus fulsiformis	6.94±0.8	7.75±0.8	0.055
Bacillus albus	9.46±1.3	9.37±1.5	0.216
Bacillus thurigiensis	9.29±1.2	9.18±1.4	0.305

Figure 1 presents the results of the daily cellulase activity of the two *Bacillus* spp. for 7 days. The activity tended to remain at the same level in the first 3 days before rising sharply to a peak on the 4th day before it declined on the 5th day (Figure 1). Thereafter, the cellulase activities began to rise again but did not attain the level of the 4th day by the 7th day when the tests terminated (Figure 1). The trends were identical for the two *Bacillus* species (Figure 1). The results in Figure 2 followed the same pattern with that of Figure 1 without any marked differences between the four *Lysinbacillus* species.

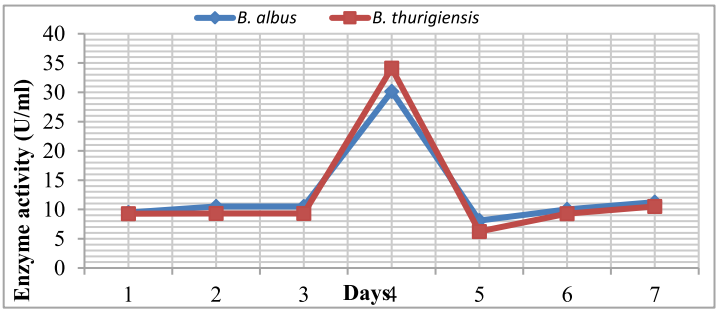

Figure 1: Trends in cellulase activities of Bacillus species isolated from coastal sediments

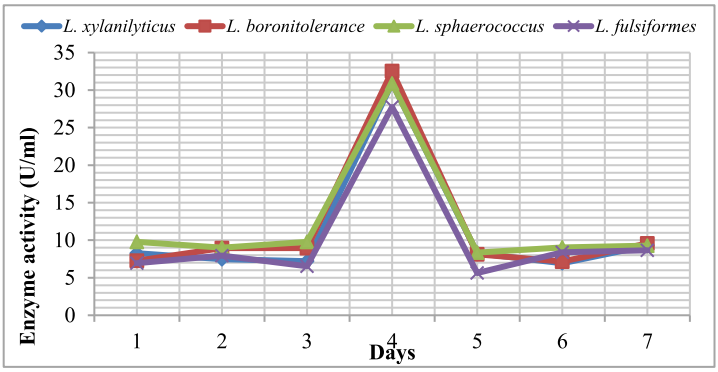

Figure 2: Trends in cellulase activities of Lysinibacillus species isolated from coastal sediments

The protein concentrations in the media markedly varied from one species to another both on day 1 and at day 4 which was the peak of cellulase activities (Table 5). The specific cellulase activities followed the same trend but with high values on day 4 which also corresponded with the peaks shown earlier in Figures 1 and 2.

Table 5: Protein concentration and specific cellulase activity of Bacillus and Lysinibacillus species after 24 h and at peak of cellulase activity

Isolates	At day 1		At day 4*	
	Protein (mg/ml)	Specific cellulase activity (U/mg)	Protein (mg/ml)	Specific cellulase activity (U/mg)
Lysinibacillus xylanilyticus	2.05	4.03	1.93	16.03
Lysinibacillus borontolerance	3.48	2.14	2.30	14.15
Lysinibacillus sphaerocus	2.63	3.72	1.78	17.35
Lysinibacillus fulsiformis	2.93	2.37	2.77	8.21
Bacillus albus	2.20	4.30	2.95	10.23
Bacillus thurigiensis	2.67	3.48	2.02	16.89

**Peak of cellulase activity (see Figures 1 and 2).*

4.0 Discussion

The results demonstrate the ability of the two *Bacillus* and four *Lysinibacillus* species to degrade wood cellulose as indicated by wood weight losses, and microchemical analysis. Bacteria are known to degrade wood in waterlogged soil, marine and terrestrial environments [11, 16-17] hence the degradation of the submerged and partially submerged wood blocks was not unexpected. Coastlines, mangroves and estuarine areas receive organic matter, including lignocellulosic wastes from rivers, surface run-offs and ocean waves. The resulting sediments become microbial "bee-hives" of nutrient recycling [20], which involves bacteria with varying cellulose-degradation potentials and cellulase enzyme activities.

The test *Bacillus* and *Lysinibacillus* species from the sediments were also able to degrade filter paper further indicating their ability to degrade cellulose irrespective of the form. In addition, degradation of filter paper suggests that they can produce the full complements of cellulases (endoglucase, exoglucanase and β-glucosidase). This is an obvious adaptation to the sediments as an ecological niche that contains lignocellulosic wastes.

The microchemical test showed that the lignin component was slightly attacked, which suggests that the organisms may be modifying the lignin barrier in order to bypass it. While lignin degradation has been mainly associated with white-rot fungi, reports that bacteria can degrade lignin are emerging [21-23]. *Bacillus* was listed as one of the organisms identified as lignolytic [22], which lends credence to the partial lignin degradation by the *Bacillus* species isolated from the coastal sediments in this study. Although studies on biodegradation of lignin has focused more on fungi with little attention to bacteria [24], suffice it to say that the lignin barrier has to be by-passed one way or the other to gain access to cellulose. In this case the cellulolytic bacteria in the marine sediments may act in consortium to synergistically degrade the lignin. This consortium approach for lignin degradation by bacteria was demonstrated in a recent study [25].

It is no surprise that cellulose-degrading *Bacillus* were found in the coastal sediment because the genus tends to be ubiquitous and can live under aerobic, anaerobic and adverse conditions which the marine environment manifests [26].

Bacillus spp have been extensively investigated for cellulase enzymes, and their enzymes have been noted for their efficacy [27]. Indeed, *Bacillus* species have been practically isolated in several studies seeking cellulose-degrading bacteria [18, 28-30]. In contrast, there are few studies on *Lysinibacillus* especially isolates from marine ecosystems. However, cellulase-producing *Lysinibacillus* species have been isolated from some coastal wetland and estuary [31, 32]. Thus, the isolation of cellulolytic *Lysinibacillus* species in the coastal sediments adds to the limited knowledge of its presence in marine ecosystems.

The cellulase and specific cellulase activities of the *Bacillus* and *Lysinibacillus* strains can be considered generally substantial especially as they were assayed in the crude state and were not produced under optimal conditions. For example, it has been reported that specific cellulase activity of *Aspergillus niger* under optimum conditions increased from 7.11 U/mg in the crude state to 484.3 U/mg after all the purification steps [33]. It is therefore expected that the cellulase activities of the *Bacillus* and *Lysinibacillus* spp. will rise above the peak values of 30.17-34.11 (cellulase activity) and 8.321-16.89 (specific cellulase activity) recorded in this study if purified. Cellulase activities vary with organisms and source of the organisms. For example, cellulase activity of a *Bacillus* strain isolated from mangrove soil was found to be 1.5100.060 U/mL of CMCase activity [34], which is lower than those of the *Bacillus* species encountered in this study.

The trends in cellulose degradation, and cellulase and specific cellulase activities generally tended to be identical for all species irrespective of the fact that they belong to two different genera and different species. This suggests a response to the same selective pressure in the marine sediments' ecological niche. It also indicates that the organisms similarly adapted to the harsh extreme conditions of the marine environment. Support for this inference comes from the finding that culturing with mineral salts medium or seawater did not lead to significant differences in cellulase activity. However, it can be argued that the identical patterns may not be unconnected with genetic/evolutionary similarities between *Bacillus* and *Lysinibacillus.* After all, *Lysinibacillus* was split from *Bacillus* not too long ago [35].

Study implication, limitations and future direction

The outcome of the study suggests that coastal sediments deserve more attention as potential sources of microorganisms that can produce cellulolytic enzymes for industrial applications. The advantage of the marine environment is that the cellulase-producing organisms developed mechanisms for survival under harsh conditions which the marine environment presents; . This attribute is advantageous in industrial applications because the catalytic power of the enzymes is likely to remain stable in a bioreactor environment. However, this study was not extended to purification of the enzymes, which would have given a better picture of the catalytic power of the enzymes. Thus, future studies will focus on the purification and optimization of the enzymes, and the assessment of the extent of the endo-glucanase, exo-glucanase and β-glucosidase activities of the *Bacillus* and *Lysinibacillus* spp.

5.0 Conclusion

The species of the genera *Bacillus* and *Lysinibacillus* isolated from the coastal sediments were able to degrade wood cellulose and filter paper with a potential to modify lignin as indicated by weight loss and microchemical analysis.

The cellulase and specific enzyme activities observed can be considered substantial because they were tested in the crude state. The observation that these marine coastal sediment bacteria were able to produce substantial levels of cellulases without optimization optimization and purification suggests that they have potential industrial applications. It was observed that the trends in the degradation of cellulose and enzyme activities did not differ markedly by genus or species. This is an indication of a convergent evolutionary response to adverse conditions which the marine environment presents; . This inference is supported by the absence of significant differences in enzyme activities when MSM was substituted with seawater while culturing the organisms for enzyme production. In conclusion, the *Bacillus* and *Lysinibacillus* species from the coastal sediments were found capable of degrading cellulose under both aerobic and anaerobic conditions; and they produced cellulolytic enzymes that can be useful in industries. Thus, researchers should give more attention to marine sediment microbes for potential biotechnological applications.

Conflict of interest

Authors declare no conflict of interest

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