



IDENTIFICATION AND APPLICATION OF DRYWOOD TERMITE (*Cryptotermes cavifrons* Banks) GUT MICROBIOTA FOR PLASTICS BIODEGRADATION

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ABSTRACT

Plastic has recently become the focus of global attention due to high accumulation in the environment which consequently threatens man and the ecosystem. This research identified dry wood termites' gut microbiota and explored their potential for plastic biodegradation. Termite samples were collected from dead and decaying woods and polythene bags from disposal sites around Dutse, Jigawa state, Nigeria. Homogenized termite's guts were cultured on Nutrient agar, incubated at 37 °C for 24 hours and biochemical tests performed on different isolates. Two plastic categories; high-density polyethylene (HDPE) and low-density polyethylene (LDPE) were cut into pieces, weighed and aseptically transferred into prepared nutrient broth with 1.0×10^5 , 1.0×10^4 , 1.0×10^3 , 1.0×10^2 and 1.0×10^1 microbial cultures while another treatment without microbes was maintained as control and plastic percentage weight loss was calculated after 30 days while analysis of variance was used to test the difference among treatments. Three bacterial species capable of degrading plastics were identified viz; *Bacillus cereus*, *Enterobacter hormaechei* and *Pseudomonas aeruginosa*. The control treatment had the lowest percentage weight loss of $1.38 \pm 0.81\%$ while in *Bacillus cereus*, the highest was $17.24 \pm 7.31\%$ in 1.0×10^5 LDPE treatment, *Enterobacter hormaechei* had the highest efficacy of $11.40 \pm 2.31\%$ in 1.0×10^5 LDPE and *Pseudomonas aeruginosa* had the highest efficacy of $5.01 \pm 3.15\%$ in 1.0×10^4 LDPE with a significant difference ($P < 0.05$) among treatments. Identified bacterial strains were more efficient on LDPE with *Bacillus* and *Enterobacter* having a linear relationship with it, giving an auspicious indication and possibility of their use in plastic clean-up.

Keywords: Plastic; Drywood termite; Gut microbiota; Biodegradation;

INTRODUCTION

Plastics are synthetic or semi-synthetic materials made from organic matter including wood, paper, wool, crude oil and other renewable raw materials (Amit *et al.*, 2022). They are mainly composed of long polymer chains with high flexibility and superior chemical stability and mechanical properties which made them desirable for a wide range of uses in many areas including agriculture, medicine, textiles, packaging,

construction etc (Yang *et al.*, 2023). Plastic products have brought great convenience to man because of low cost and easy production but recently they have become the focus of global attention due to high accumulation in the environment which consequently threatens man and the ecosystem. The annual plastic consumption has exceeded 300 million tonnes which continues to grow exponentially and only 10% of which are recycled, 12% are burned while over 70% are discarded into the soil,

air and sea (An *et al.*, 2023). The most common disposal methods are landfilling and incineration which have adverse implications on the environment especially the ones that have nanocomposite polymers. Plastic pollution constitutes aesthetic nuisance, reduces soil permeability and productivity (Attila *et al.*, 2024), it breaks down into micro plastics, microfibers, toxic chemicals and organic pollutants that enter the food chain and cause serious damage to living creatures (Ziani *et al.*, 2023), affects reproductive success and alter enzymatic processes (Alimba and Faggio, 2019). Plastics consist of numerous compounds including several chemicals, basic among which are monomers, oligomers, polymers and additives such as plasticizers, antioxidants, heat stabilizers and pigments as well as other chemical pollutants adsorbed from the environment which make plastics a hub for toxic substances (Cai *et al.*, 2023). Plastic share some physical and chemical properties with lignocellulose polymers including a carbon skeleton, a hydrophobic nature and amorphous/crystalline regions (Al-Tohamy *et al.*, 2023) and can be degraded by mechanical, photochemical, thermal, and biochemical mechanisms in the natural environment (Yang *et al.*, 2023).

Cryptotermes cavifrons Banks known popularly as the drywood termite is a common pest of hardwood and other household furniture that is widely distributed all over the world (Himmi *et al.*, 2021). They are social insects that live in colonies with complete life cycle and different castes having powerful mouthparts including soldiers, sterile workers and reproductives (Scheffrahn, 2008). They're the major soil insects capable of metabolizing cellulose and are commonly found in plastic polluted sites which made them develop the ability to degrade plastics using their gut microbiota (Amit *et al.*,

2022). Termite guts reportedly contain few and highly specialized microbial species including flagellated protists, yeasts, and bacteria that break down complex polymers such as cellulose, hemicellulose, lignin and plastic into simpler forms (Janayita *et al.*, 2024). These microbes produce an array of enzymes that facilitate the digestion of polymers into CO₂ and water as final products of biodegradation, which has the advantages of low energy consumption and green environmental protection though it is extremely slow for major plastics (Akram *et al.*, 2024). Many bacterial species including *Pseudomonas* strain, *Escherichia*, and *Bacillus* isolated from landfills have proved to be capable of decomposing plastic and utilizing it by secreting enzymes that hydrolyse large polymer chains into smaller units which are utilized as carbon sources for the microbes (Zhang *et al.*, 2023). The type of plastic whether its low or high density determines to a very large extent its susceptibility to biodegradation, hence this research was carried out to identify dry wood termites' gut microbiota and explore their potential for low and high density plastics biodegradation.

MATERIALS AND METHODS

Sample Collection

All glasswares and other utensils were thoroughly washed with water and detergent, rinsed with distilled water and air dried after which they were sterilized in a hot air oven at 160⁰C for one hour according to Ajayi *et al.* (2016). Purposive sampling strategy was employed where drywood termite (*Cryptotermes cavifrons* Banks) samples were collected from their nests around dead and decaying woods using transect divided into 6 uniform spaced sections in order to get representative coverage of all termite castes according to Dambros *et al.* (2020). Used plastic bags

were collected from disposal sites around Dutse central market in Jigawa state, Nigeria using stainless steel tong and transferred into glass containers according to Oju *et al.* (2023) while all the collected samples were transported to the laboratory for preparation and analysis.

Plastic Separation and Preparation

Plastic samples were thoroughly washed with tap water until no dirt or debris was visible on them and the samples were air-dried at room temperature before being sorted and categorized into high-density polyethylene (HDPE) and low-density polyethylene (LDPE) which were cut into small pieces of about 1 cm², surface sterilized with 70% ethanol solution and washed with distilled water according to Evdokia *et al.* (2019) and Dey *et al.* (2023).

Termites Guts' Dissection and Microbial Isolation

Termites were washed with sterile distilled water, dried and outwardly sterilized using 70% ethanol according to Saha *et al.* (2022) after which the guts were extracted with forceps, crushed and homogenized with sterile distilled water. An aliquot of 0.1 ml was cultured on Nutrient agar, incubated at 37 °C for 24–48 hours, total bacterial count was done using plate count method while sub-culturing of each isolates was done until pure colonies were obtained and their morphology was observed according to Ajiboye and Emmanuel (2021). Bacterial isolates were identified using Gram staining technique and biochemical tests were performed on different isolates according to Bate *et al.* (2023).

Plastic Degradation Test

The sterile pre-weighed high-density polyethylene (HDPE) and low-density polyethylene (LDPE) were aseptically

transferred into 250 ml sterile conical flasks along with 200 ml nutrient broth and 1.0×10⁵, 1.0×10⁴, 1.0×10³, 1.0×10² and 1.0×10¹ bacterial cultures were added to each flask while another treatment without microbes was maintained as control according to Thanga *et al.* (2016). Flasks were incubated at 37 °C for 30 days, plastics were washed and dried before being weighed again and the degradation potential of different microbial isolates were determined by calculating the percentage plastic weight loss using the formula:

$$\text{Percentage weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\% \quad 1.$$

Statistical Tests

One way analysis of variance (ANOVA) was used to test if plastic degradation capacity was significantly different among different counts of microbial cultures while Pearson correlation was conducted to determine the relationship between microbial and plastic degradation.

RESULTS

Identification of Drywood Termites' Gut Microbiota

There were three identified microbial species in the gut of drywood termite during this research, all of which are bacteria viz; *Bacillus cereus*, *Enterobacter hormaechei* and *Pseudomonas aeruginosa* all of which are rod shaped and motile.

Microbes' Plastic Degradation Potentials

The lowest plastic percentage weight loss was 1.38±0.81% in the control. In *Bacillus cereus*, the highest percentage weight loss was 17.24±7.31% in 1.0×10⁵ LDPE treatment with its highest HDPE degradation being 3.21±1.80 in 1.0×10⁵ which was

significantly lower ($P < 0.05$). Both LDPE and HDPE had positive linear relationship with microbial count, having r -values of 0.82 and 0.61 respectively. Table 1 presents

the plastic degradation performance of *Bacillus cereus* on HDP and LDP while table 2 is a correlation matrix showing the respective r -values.

Table 1: Degradation Rate of Plastic Treated with *Bacillus cereus* from Termite Gut

Microbial count (CFU)	0	1.0×10^1	1.0×10^2	1.0×10^3	1.0×10^4	1.0×10^5	F-value	P-test
HDPE Degradation (%)	1.38±0.81	1.72±0.11	1.54±0.10	3.11±0.51	2.58±0.21	3.21±1.80	2.66	$P < 0.05$
LDPE Degradation (%)	1.38±0.81	5.68±1.21	6.21±1.13	8.74±1.15	11.71±1.22	17.24±7.31	1.79	$P < 0.05$

Table 2: Correlation Matrix Showing *Bacillus cereus* Count and Plastic degradation Relationship

Name	Microbial count	HDPE Degradation	LDPE Degradation
Microbial count	1		
HDPE Degradation	0.61	1	
LDPE Degradation	0.82	0.85	1

Enterobacter hormaechei also had its highest plastic degradation as 11.40±2.31% of LDPE in 1.0×10^5 which shows continuous relationship while on HDPE the highest degradation was 2.72±1.30% in 1.0×10^3 count and the r -values for LDPE

and HDPE relationships with microbial count are 0.62 and -0.23 respectively. Plastic degradation performance of *Enterobacter hormaechei* on HDP and LDP is presented in Table 3 while their r -values are shown in table 4.

Table 3: Degradation Rate of Plastic Treated with *Enterobacter hormaechei* from Termite Gut

Microbial count (CFU)	0	1.0×10^1	1.0×10^2	1.0×10^3	1.0×10^4	1.0×10^5	F-value	P-test
HDPE Degradation (%)	1.38±0.81	1.55±0.21	1.61±0.18	2.72±1.30	1.82±0.15	1.95±0.11	4.17	$P < 0.05$
LDPE Degradation (%)	1.38±0.81	5.21±0.52	7.11±0.84	8.64±1.02	9.72±1.01	11.40±2.31	3.89	$P < 0.05$

Table 4: Correlation Matrix Showing *Enterobacter hormaechei* Count and Plastic Degradation Relationship

Name	HDPE Degradation	Microbial count	LDPE Degradation
HDPE Degradation	1		
Microbial count	-0.23	1	
LDPE Degradation	-0.01	0.62	1

The highest plastic degradation by *Pseudomonas aeruginosa* was 5.01±3.15% in 1.0×10^4 LDPE treatment while in HDPE it was 3.11±1.10% in 1.0×10^3 treatment with no linear increase in both plastic types. The

r -value of the relationship between *Pseudomonas aeruginosa* count and LDPE degradation was -0.04 and that of HDPE was -0.06. The degradation performance of *Pseudomonas aeruginosa* on HDP and LDP

are presented in Table 5 while their relationship with *Pseudomonas aeruginosa*

was shown in table 6.

Table 5: Degradation Rate of Plastic Treated with *Pseudomonas aeruginosa* from Termite Gut

Microbial count (CFU)	0	1.0×10 ¹	1.0×10 ²	1.0×10 ³	1.0×10 ⁴	1.0×10 ⁵	F-value	P-test
HDPE Degradation (%)	1.38±0.81	2.14±0.21	1.83±0.16	3.11±1.10	1.98±0.14	2.01±0.11	3.61	P < 0.05
LDPE Degradation (%)	1.38±0.81	2.05±0.42	3.02±0.19	2.11±0.96	5.01±3.15	2.32±0.25	2.72	P < 0.05

Table 6: Correlation Matrix Showing *Pseudomonas aeruginosa* Count and Plastic Degradation Relationship

Name	Microbial count	HDPE Degradation	LDPE Degradation
Microbial count	1		
HDPE Degradation	-0.06	1	
LDPE Degradation	-0.04	0	1

DISCUSSION

The identified bacterial species form part of the normal gut flora of many cellulolytic organisms including flies and termites (Ramin *et al.*, 2009), they have the ability to produce enzymes such as Cutinase, Lipase, PETase etc that are associated with the degradation of plastics (Mohan *et al.*, 2020). Bacterial species of *Pseudomonas*, *Bacillus* and *Acinetobacter* that can degrade plastics and petroleum hydrocarbons were found in the Persian Gulf region and could be ubiquitous in the future because of the widespread nature of plastics (Johnson, 2024).

Bacillus cereus is a large Gram-positive spore producing bacterium capable of depolymerizing alkali lignin effectively by producing lignin peroxidase and laccase and it was found to effectively degrade alkali lignin by about 26.72% (Yang *et al.*, 2021). Other researchers reported *B. Cereus* to be capable of growing in plastic polluted sites and degraded up to 14% over a 42 day trial (Vethanayaham *et al.*, 2024). The bacterium's increased plastic

degradation performance with increase in its count and the very high positive correlation is a pointer to its optimum value and shows its potential for large scale industrial application which is badly needed to salvage the environment from the menace of plastic pollution (Ganbold *et al.*, 2023).

The bacterium *Enterobacter hormaechei* is a Gram negative and facultative anaerobic species that has the capability to utilize diverse plastic polymers as their sole source of carbon (Wenxiao *et al.*, 2024). *E. hormaechei* degraded LDPE with a significant continuous relationship compared to HDPE which could be attributed to HDPE's resistance to tear and abrasion along with higher shear and tensile strength (Mohan and Venkata, 2014). This implies that exploring the large scale application of *E. hormaechei* in plastic degradation may only be feasible in LDPE which constitutes only about 36% of plastic wastes (Gwada *et al.*, 2018).

Pseudomonas aeruginosa is a Gram-negative, aerobic and free living bacteria that can depolymerize several plastic types

such as polyvinyl chloride, polyurethane etc (Mahesh *et al.*, 2024). The non-linear and negative correlation between *P. aeruginosa* and both plastic types in this research could be attributed to other factors besides the bacterial population being responsible for plastic degradation. This finding is similar to that of Lee *et al.* (2020) who found an inverse relationship between the growth rates of *P. aeruginosa* and biodegradation rates and they concluded that composition and properties of intermediate molecules produced during plastic biodegradation affects the degradation rate.

CONCLUSION

This research identified three bacterial species; *Bacillus cereus*, *Enterobacter hormaechei* and *Pseudomonas aeruginosa* capable of degrading plastics in the gut of drywood termite (*Cryptotermes cavifrons* Banks). The lowest percentage plastic weight loss was $1.38 \pm 0.81\%$ in the control while the highest was $17.24 \pm 7.31\%$ in 1.0×10^5 LDPE treatment with a significant difference among treatments in all microbes and significantly higher degradation rate in LDPE than HDPE. *B. cereus* and *E. hormaechei* had positive linear relationship with plastic degradation rates, indicating their potential for large scale application.

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