Molecular characterization and cellulolytic activities bacterial isolates from the hindgut of wood-feeding termites *Amitermes evuncifer* Silvestri

ABSTRACT

Aims: To characterize the bacterial microbiota of the wood-eating termite *Amitermes evuncifer* Silvestri using the 16S rRNA molecular analysis and to determine the cellulolytic capability of the isolates.
Study design: The study was designed to assess the different types of cultivable bacteria present in the gut of *A. evuncifer* using. The cellulolytic potential of the bacterial isolates was also determined.
Place and Duration of Study: Department of Microbiology, Ekiti State University Ado Ekiti, Nigeria, between January 2017 and February 2018.
Methodology: Gut microbiota of twenty wood-feeding worker termites *Amitermes evuncifer* were characterized using phenotypic methods and 16SrRNA molecular analysis. The bacterial isolates were also analyzed for cellulase production.
Results: The molecular analysis revealed that the isolates belonged to the Bacteria domain and phyla *Pseudomonas, Bacillus, Achromobacter and Lysinibacillus.* All the bacterial isolates exhibited both exoglucanase (FPase) and endoglucanase (CMCase) activities. Three of the isolates: *Bacillus cereus* ATCC 14579, *Bacillus mycoides* ATCC 6462 *and Pseudomonas aeruginosa* ATCC 10145 exhibited the highest endoglucanase activity of 6.38 µmol $min^{-1}mg^{-1}$, 5.96 µmol $min^{-1}mg^{-1}$ and 4.89 µmol $min^{-1}mg^{-1}$ respectively. Meanwhile *Pseudomonas aeruginosa* ATCC 10145 showed the highest exoglucanase activity (1.47 µmol $min^{-1}mg^{-1}$) followed by *Bacillus cereus* ATCC 14579 (1.14 µmol $min^{-1}mg^{-1}$) and *Pseudomonas aeruginosa* DSM 50071 (1.10 µmol $min^{-1}mg^{-1}$).
Conclusion: Diversity of cellulolytic bacterial isolates was found in the hindgut of *A. evuncifer*. Further work needs to be carried out to determine the properties of the cellulase produced by bacterial community and the possible application of the isolates and their extracellular enzymes in biodegradation and treatment of agricultural wastes to alleviate pollution.

Keywords: Cellulase, Endoglucanase, Exoglucanase, Pseudomonas, Bacillus, Achromobacter

1. INTRODUCTION

Termites are detritivores consuming dead plants at any level of decomposition. They thus play a vital role in the ecosystem by recycling waste material such as dead wood, faeces and plants [1, 2]. Termites are a group of eusocial insects that harbor a consortium of aerobic, anaerobic, and microaerophilic bacteria that are responsible for the degradation of cellulose and hemicellulose and benefit their host organism [3]. Termites' gut microbiota are exchanged between colony members and transferred to the next generation through trophallaxis [4].
Various groups of insects have developed symbiotic relationships with fungi; notable examples in three different insect orders include termites, ants, and ambrosia beetles. Interactions associated with termites and fungi have been reported in both lower and higher termites [5].
The macrotermitinae comprises of the economically important termite species [6] that have been comprehensively studied [7]. Considering the plant-biomass degradation and nitrogen metabolism in termites, the presence of diverse bacterial

lineages in termites [8] and the evolutionarily diverse termite species, the termite gut microbial populations are of significant interest and need to be isolated and characterized in order to better understand the termite symbiotic systems.
In the higher termites, the fungus-growing termites (Subfamily Macrotermitinae, Family Termitidae) have evolved symbiotically with the fungus Termitomyces [9]. The macrotermitine termites are distributed throughout the tropical and subtropical areas of Asia and Africa and have over 330 species belonging to 12 genera [9].
Previous work by Femi-Ola *et al.* [10] on the characterization of bacterial isolates in the hindgut of *Amitermes evuncifer* was based on cultural and biochemical characterization. The objective of this study was to characterize the bacterial microbiota of the wood-eating termite *Amitermes evuncifer* Silvestri using the 16S rRNA molecular analysis and to determine the cellulolytic capability of the isolates.

2. MATERIAL AND METHODS

2.1 Collection of termites

Twenty wood-eating worker termites *Amitermes evuncifer* were collected from infested planks on the campus of Ekiti State University Ado Ekiti. The termite samples were identified as *Amitermes evuncifer* of the order Isoptera family Termidae at the Department of Zoology, Ekiti State University Ado Ekiti, Nigeria.

2.2 Assessment of microflora in the hindgut of *A. evuncifer*

Termite samples were surface sterilized in ethanol (98%). Thereafter they were rinsed in two changes of sterile distilled water. Each of the termites was aseptically dissected to remove the hindgut, which was then placed into 10 ml sterile normal saline solution. The gut sample was crushed with a sterile glass rod to release the intestinal contents. Serially diluted suspension was plated on nutrient agar plates in replicates and incubated at 35^{o}C for 48 hours. Pure cultures of the isolates were obtained by sub culturing serially onto Nutrient agar plates.

2.3 Phenotypic Characterization of bacterial isolates

Pure cultures of the isolates were identified based on their cultural, morphological and physiological characteristics in accordance with the taxonomic scheme of Barrow and Feltham [11] and reference to Holt *et al.* [12].

2.4 Molecular characterization of bacterial isolates

2.4.1 Genomic DNA (gDNA) extraction and PCR Amplification

Bacterial isolates were separately inoculated into sterile falcon tubes containing 15 ml Luria Bertani (LB) medium and incubated for 48hr in a shaking incubator (at 150 rpm) at 37°C. The cultures were then centrifuged at 6000 rpm for 10 min. The resultant pellets were separately re-suspended in sterile micro tubes containing 0.2 ml of tris-ethylene diamine tetra acetic acid (TE) buffer (10 mM TrisHCl, 1 mM EDTA, pH 8.0).
The cell suspensions were used for gDNA extraction using the Ultraclean® Mega soil DNA isolation kit (MO BIO Laboratories, Inc.) according to the manufacturer's protocol. Purified DNA was quantified photo metrically (NanoDrop; Thermo Fisher Scientific, Germany) and used as a template for amplification of 16S rRNA genes using the universal bacterial primers (8F 5'-AG (A/G) GTTTGATCCTGGCT-3' forward primer and 1492R 5' CGGCTACCTTGTTACGACTT-3' reverse primer) according to their position in relation to the *Escherichia coli* gene sequence [13].
For each PCR, 1 µl (25 ng/ µl) of the template was mixed with TaKaRa Ex Taq™ HS (5 units/µl) according to the manufacturer's protocol. The PCR conditions were as described by Mackenzie *et al.* [14] except the final extension was at 72°C for 8 min. PCR product size was checked using a 1% agarose gel stained with ethidium bromide. The amplicons were gel purified using Macherey-Nagel NucleoSpin extract II kit as per the manufacturer's protocol and eluted in 30 µl of TE Buffer (5 mM, pH 8.0).

2.4.2 DNA Sequencing and Phylogenetic Analysis

Sequencing of the purified PCR products was done using a commercial service provider (Macrogene, South Korea). Sequences of the isolates were manually edited in chromas and checked for presence of artifacts or chimeric structures using the Mallard software [15]. A search for similar sequences using BLASTN program was performed, and sequence alignment was performed using the CLUSTAL Omega program (http://www.clustal.org). A neighbour-joining tree of the aligned sequences was constructed using MEGA V5.10 [16].
Evolutionary distances were computed using the Maximum Composite Likelihood method [17]. To obtain support values for the branches, bootstrapping [18] was conducted with 1000 replicates. All sites, including gaps in the sequence alignment, were excluded pair wise in the phylogenetic analysis. Using the resultant neighbour-joining tree, each isolate was assigned to the proper taxonomic group. The taxonomic assignment was confirmed at a 90% confidence level using the naïve Bayesian rRNA classifier on the RDP website [19]. All sequences were deposited in Gen Bank nucleotide database.

2. 5. Physiological studies of bacterial isolates

Bacterial isolates were screened for their endoglucanase and exoglucanase activities using carboxymethylcellulose (CMC) and Filter paper as the substrate respectively.

2.5.1 Screening for cellulase-producing bacteria

The agar diffusion method was employed to screen for cellulolytic abilities of the bacterial isolates. Pure cultures of bacterial isolates were individually transferred to Carboxymethylcellulose CMC agar plates. After incubation for 48 hours,

CMC agar plates were flooded with 1 % Congo red and allowed to stand for 15 min at room temperature. One molar NaCl was thoroughly used for counterstaining the plates [20]. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the largest clear zone were selected for cellulase production.

2.5.2 Cellulase production by cellulase-producing bacteria

Cellulase production was carried out by submerged fermentation procedures in accordance with the method of Sharma and Bajaj [21] with slight modification, using basal salt medium containing 1% (w/v) CMC as carbon sources. The bacterial isolate was inoculated into the basal salt medium supplemented with CMC and incubated for 48h. Two milliliters of the pre-inoculated culture was inoculated into 100 mL of Mandels Salt Medium [Urea 0.3 g L^{-1}, $(NH_4)_2SO_4$ 1.4 g L^{-1}, KH_2PO_4 2.0 g L^{-1}, $CaCl_2$ 0.3 g L^{-1}, $MgSO_4$ 0.3 g L^{-1}, yeast extract 0.25g L^{-1} and peptone 0.75 g L^{-1} with 10 g L^{-1} of Carboxymethylcellulose (CMC) in 0.05M citrate buffer (pH 6.0)] as production medium contained in Erlenmeyer flasks (250mL) and incubated on a rotary shaker 200 rpm at 45^{o}C. The enzyme activity was assayed periodically at different time intervals using the method described by Ghose [22]. Two milliliters of the fermented broth was centrifuged at 10,000× g for 5 min at 4^{o}C to obtain the supernatant and used as the crude extract for cellulase assay after appropriate dilutions [23].

2.5.3 Determination of protein content

Protein concentration was determined using the Lowry method [24]. The reagents used include; Reagent A: 2% $NaCO_3$ in 0.1 N NaOH; Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% Na or K tartarate; Reagent C: 100ml of Reagent A + 2ml of reagent B and Reagent E: 1:2 dilution of John's reagent water. Graded concentrations of Bovine Serum Albumin (BSA) in tubes were prepared. Then 0.3 ml of each concentration was measured into test tubes, 3 ml of reagent C was added, mixed and left for 10 min. Then 0.3ml of reagent E was added, mixed and left for 30 min. The optical density was read at 600nm. The graph of OD versus concentration of BSA and used as standard curve of BSA. The same was read for unknown substance and the protein concentrations from the standard curve are read off and obtained by multiplying with dilution factor. All readings were obtained in triplicates.

3. RESULTS AND DISCUSSION

Molecular identification of the isolates

A total of 10 pure representative isolates from guts of *Amitermes evuncifer*, were selected based on their morphological and biochemical characteristics for further characterization using molecular approach. The bacterial isolates showed various colonial appearances on nutrient agar ranging from smooth surfaces, raised elevation, circular shaped, mucoid colony, pigmented, translucent, opaque, shiny colony and large, medium colonies. Five of the bacterial isolates were Gram positive bacilli, while the remaining five were Gram negative bacilli (Table 1, 2). Moreover, three pigmented bacterial isolates that appeared as Gram negative rods were positive for catalase, oxidase and urease production. The results satisfy the criteria for the genus *Pseudomonas* [11, 12]. Among the ten isolates, five exhibited characteristics that were similar to the genus Bacillus as described by Bergey's manual of determinative bacteriology [12].

The 16SrRNA gene amplified products from the ten (10) bacterial isolates were successfully sequenced. Phylogenic analysis using BLAST software (http://www.ncbi.nih.gov) showed that they belong to the domain Bacteria and phyla *Pseudomonas, Bacillus, Achromobacter and Lysinibacillus.* The isolates comparison to known sequences in the Gen bank database indicated sequences similarities up to 91% with known sequences. Some of the isolates were closely affiliated with members of the genus *Pseudomonas* DSM 50071 (93%), *Bacillus cereus* ATCC 14579 (95%), *Bacillus mycoides* ATCC 6462 (95%), *Pseudomonas aeruginosa* NBRC 12689 (94%), *Achromobacter insuavis* LMG 26845 (91%), *Bacillus cereus* 14579 (95%), *Lysinibacillus fusiformis* NBRC 15717 (95%), *Pseudomonas aeruginosa* ATCC 10145 (96%), *Bacillus anthracis* strain ATCC 14578 (96%), *Achromobacter insolitus* LMG 6003 (95%) sequence identity (Table 3). Femi-Ola *et al.* had earlier reported the colonization of the hindguts of *Amitermes evuncifer* by a diverse group of bacteria such as the species of *Bacillus, Micrococcus, Serratia* and *Streptococcus* [10]. Several authors have reported similar organisms in the gut of termites [25, 26, 27].

Both the phylogeny and the sequencing showed that members of the same species in the termite are closely related and share a greater proportion of phylogenetic interconnectedness than bacteria of different species in the same termite. These patterns of association suggest that community differences among members of the Macrotermitinae may be shaped by co-diversification with their termite host. However, this pattern could also arise as a product of termite species with similar ecologies acquiring similar gut microbes [28].

This observations correlates with the findings of previous investigators [8, 25, 29, 30], indicating that the predominance of these groups is a general trend in the Macrotermitinae. Their work showed that wood eating lineages of higher termites (Nasutitermitinae and Termitinae), are implicated in the separation of the gut communities of the termite from all other subfamilies and to the similarities between fungus-growing termites and cockroaches [30].

Table 1: Morphological characteristics of Bacterial isolates from the Gut of *Amitermes evuncifer*

Isolates	Cell shape	Colony morphology	Colour	Pigment	motility
A	Rod	Smooth edges, convex, mucoid	Greenish yellow	+	+
B	Rod	Circular, smooth, flat	White	-	+
C	Rod	Irregular, wavy, granular	Grey-white	-	+
D	Rod	Rhizoid, opaque, flat	Cream	-	-
E	Rod	Irregular, smooth, dry	Red-brown	+	+
F	Rod	Irregular, wavy, granular	Grey-white	-	+
G	Rod	Irregular, mucoid, smooth	Bluish-green	+	+
H	Rod	Irregular, flat, mucoid	Cream	-	-
I	Rod	Flat, slightly convex, smooth	White	-	+
J	Rod	Circular, opaque, smooth,	Buttery	-	-

Table 2: Biochemical Characteristics of Bacteria isolated from the gut of *Amitermes evuncifer*

Isolate	Gram stain	Spore stain	Catalase	Oxidase	MR	VP	Citrate	Urease	Indole	Identity
A	-	-	+	+	-	-	+	+	-	*Pseudomonas* sp.
B	+	+	+	-	-	+	+	-	-	*Bacillus* sp.
C	+	+	+	-	-	+	+	-	-	*Bacillus* sp.
D	-	-	+	+	+	+	+	+	-	*Pseudomonas* sp.
E	+	+	+	-	-	-	+	+	-	*Bacillus* sp.
F	+	+	+	-	-	+	+	-	-	*Bacillus* sp.
G	-	-	+	+	-	-	+	+	-	*Pseudomonas* sp.
H	-	-	+	-	-	+	-	-	+	*Achromobacter* sp.
I	+	+	+	-	-	+	+	-	-	*Bacillus* sp.
J	-	-	+	-	-	-	+	+	-	*Achromobacter* sp.

(+) indicates positive results or presence of, and (-) a negative results/ no presence.

MR- Methyl Red; VP- Voges Proskauer

Table 3: Molecular analysis of bacterial isolates

Isolate	Similar strains	Accession no	Similarity (%)
A	*Pseudomonas* DSM 50071	NR_117678.1	93
B	*Bacillus cereus* ATCC 14579	NR_074540.1	95
C	*Bacillus mycoides* ATCC 6462	NR_115993.1	95
D	*Pseudomonas aeruginosa* NBRC 12689	NR_113599.1	94
E	*Bacillus cereus* 14579	NR_074540.1	95
F	*Lysinibacillus fusiformis* NBRC 15717	NR_112569.1	94
G	*Pseudomonas aeruginosa* ATCC 10145	NR_114471.1	96
H	*Achromobacter insuavis* LMG 26845	NR_117706.1	91
I	*Bacillus anthracis* ATCC 14578	NR_041248.1	96
J	*Achromobacter insolitus* LMG 6003	NR_025685.1	95

Phylogenetic Analysis

The phylogenetic tree based on the 16S rRNA sequences of bacteria species from wood termite gut is presented in Figure 1. Sequences aggregated into four bacterial phyla (*Achromobacter*, *Pseudomonas, Bacillus* and *Lysinbacillus*) and nine clusters in conformity with the bacterial classes *Achromobacter insuavis* LMG 26845*, Achromobacter insuavis* LMG 6003*, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Lysinbacillus fusiformis, Bacillus mycoides, Bacillus anthracis* and *Bacillus cereus.*

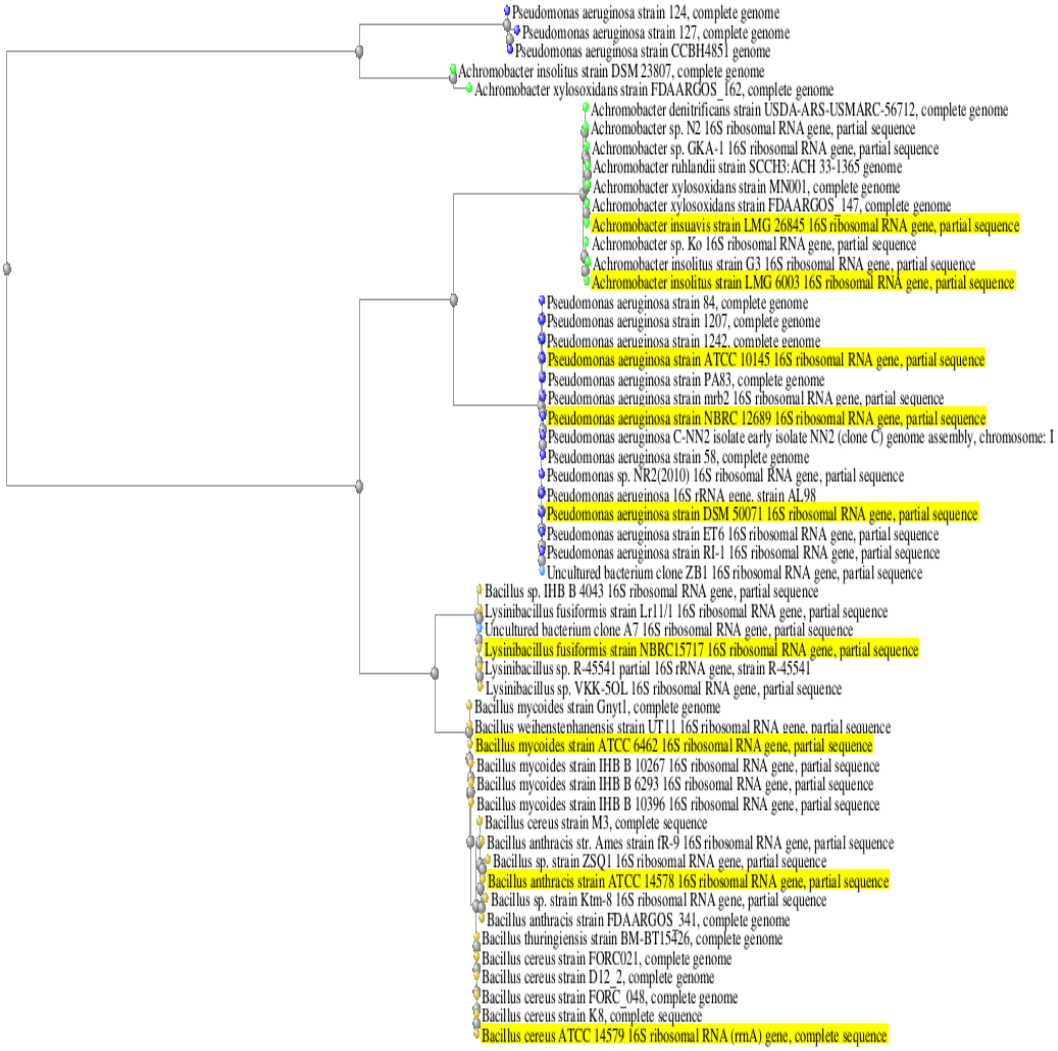

Figure 1: Phylogenetic relationship of the bacteria isolated from the hindgut of wood-eating termites *Amitermes evuncifer* Silvestri

Cellulolytic activities of bacterial isolates

All the bacterial isolates demonstrated cellulolytic activities at varying degrees. The specific activities of the exoglucanase produced by the gut bacteria ranged from 0.43 µmol $min^{-1}mg^{-1}$ to 1.47 µmol $min^{-1}mg^{-1}$ (Table 4). Meanwhile the endoglucanase activities of the isolates ranged from 2.32 µmol $min^{-1}mg^{-1}$ to 6.38 µmol $min^{-1}mg^{-1}$. The specific activity of *Pseudomonas aeruginosa* NBRC 12689 exoglucanase was the highest, while *Bacillus cereus* ATCC 14579 endoglucanase had the highest specific activity. *Lysinibacillus fusiformis;* previously known as *Bacillus licheniformis* have been isolated from termite gut by several authors and its cellulolytic activity have also been reported [31, 32, 33]. The function of the termite gut microbiota ranges from fermentative metabolism, production of acetate and other products by fermentation of mono- and oligosaccharides, while some are homoacetogenic [34, 35, 36], to reductive acetogenesis from H_2 + CO_2 [37] but this processes are still unclear [4, 38]. Diet has been shown to affect the gut communities of wood-feeding termites [39, 40]. It is believed that a complete degradation of ingested wood materials by the termite is achievable by a symbiotic interaction among these microorganisms within its gut [5].

Although the role of the bacterial community in the breakdown of lignocellulose in the termite *Amitermes* are still under study, research have shed some light on the possible contributions by bacteria to cellulose digestion in wood-feeding termites [20, 37, 41].

207 **Table 4: Summary of Endoglucanase and Exoglucanase Production by Bacterial isolates from the Gut of Amitermes evuncifer**

208

Isolates	Endoglucanase		Exoglucanase	
	Activity (µmol min^{-1})	Specific activity (µmol $min^{-1}mg^{-1}$protein	Activity (µmol min^{-1})	Specific activity (µmol $min^{-1}mg^{-1}$protein
Pseudomonas aeruginosa DSM 50071	31.18	2.83	12.15	1.10
Achromobacter insuavis LMG 26845	73.46	3.94	10.83	1.02
Bacillus cereus 14579	80.6	4.51	19.03	1.06
Bacillus mycoides ATCC 6462	94.6	5.96	16.38	1.08
Pseudomonas aeruginosa NBRC 12689	34.61	2.71	18.76	1.47
Bacillus cereus ATCC 14579	81.39	6.38	14.53	1.14
Pseudomonas aeruginosa ATCC 10145	85.62	4.89	17.18	0.85
Bacillus anthracis ATCC 14578	96.19	4.84	16.65	0.70
Achromobacter insolitus LMG 6003	49.68	2.67	7.93	0.43
Lysinibacillus fusiformis NBRC 15717	34.61	2.323	8.19	0.73

4. CONCLUSION

This study reports the molecular characterization of bacteria found in the gut of a wood-eating *Amitermes evuncifer*. The bacterial isolates were found to be cellulolytic. Further work needs to be carried out to determine the properties of the cellulase produced by bacterial community and the possible application of the isolates and their extracellular enzymes in biodegradation and treatment of agricultural wastes to alleviate pollution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chaturvedi V, Verma P. An overview of key pre-treatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. *Biotechnol.* 2013; 3:415- 431.
2. Sanderson MG. Biomass of termites and their emissions of methane and carbon dioxide: A global database. *Global Biogeochemical Cycles.* 2011; 10(4):543–557.
3. Bignell DE. Morphology, physiology, biochemistry and functional design of the termite gut. In: *Biology of Termites: A Modern Synthesis* (Bignell DE, Roisin Y, Lo N.), Pp. 375–412, 2011.
4. Brune A, Ohkuma M. Role of the termite gut microbiota in symbiotic digestion. In: *Biology of Termites: A Modern Synthesis* (Bignell DE, Roisin Y, Lo N. eds) Springer, Pp. 439-475, 2011.
5. Nobre T, Rouland-Lefevre C, Aanen DK. Comparative biology of fungus cultivation in termites and ants. In: *Biology of Termites: A Modern Synthesis* (Bignell D.E, Roisin, Y, Lo N, eds), pp. 193–210, 2011.
6. Ahmed BM, Nkunika POY, Sileshi WG, French JRJ, Nyeko P, Jain S. Potential impact of climate change on termite distribution in Africa. *Brit. J. Environ. Clin. Change.* 2011; 1:172-189.
7. Mathew GM, Ju YM, Lai CY, Mathew DC, Huang CC. Microbial community analysis in the termite gut and fungus comb of *Odontotermes formosanus*: the implication of *Bacillus* as mutualists. *FEMS Microb. Ecol.* 2012; 79:504-517.
8. Makonde HM, Mwirichia R, Osiemo Z, Boga HI, Klenk HP. 454 Pyrosequencing-based assessments of bacterial diversity and community structure in termite guts, mounds and surrounding soils. *Springer Plus.* 2015; 4:471.
9. Aanen DK, Ros VID, de-FineLicht HH, Mitchell J, Boomsma JJ. Patterns of interaction specificity of fungus-growing termites and Termitomyces symbionts in South Africa. *BMC Evol Biol.*2007; 7:115–126.
10. Femi-Ola TO, Adewumi KB, Oye-Bamgbose O, Aderibigbe EY. Incidence of *Micrococcus luteus* in the gut of wood-eating termites (*Amitermes evuncifer). Afr. J. Sci.* 2001; 4(1): 62-67.
11. Barrow GI, Feltham RKA. Cowan and Steel's manual for identification of Medical Bacteria. 3rd Edition Cambridge University Press London, 1993.
12. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. *Bergey's Manual of Determinative Bacteriology*. 9th edition. Baltimore, Williams and Wilkins, 1994.
13. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt *et al*. (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp115-175, 1991.
14. Mackenzie LM, Muigai AT, Osir EO, Lwande W, Keller M, Toledo G, Boga HI. Bacterial diversity in intestinal tract of fungus cultivating termite *Macrotermes michaelseni* (Sjöestedt). *Afr. J. Biotechnol*. 2007; 6:658-667.
15. Ashelford KE, Chuzhanova NA, Fry JC, Jones AJ, Weightman AJ. New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. *Appl. Environ. Microbio.* 2006; 72:5734-5741.
16. Tamura K, Peterson D, Peterson N, Stecher C, Nei M, Kumar S. MEGA 5: Molecular evolutionary genetic analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 2011; 28(10): 2731-2739.
17. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci.* U. S. 2004; 101:11030–11035.
18. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution.* 1985; 39:783-791.
19. Cole JR, Chai B, Farris RJ, Wang Q, Kulam SA, McGarrell DM, Garrity GM, Tiedje JM. The Ribosomal Database Project (RDP-II): Sequences and tools for high-throughput rRNA analysis. Nucleic *Acids Res*. 2005; 33:294-296.

20. Teather RM, Wood PJ. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl. Environ. Microbio.* 1982; 43:777-780.
21. Sharma P, Bajaj BK. Production and partial characterization of alkali-tolerant xylanase from an alkalophilic *Streptomyces* sp. CD3. *J. Sci. Ind. Res*. 2005; 64:688-697.
22. Ghose, T. K. (1987). Measurement of cellulase activities. *Pure and Appl. Chem*. *J.* 59:257-268.
23. Mmango-Kaseke Z, Okaiyeto K, Nwodo UU, Mabinya LV, Okoh AO. Optimization of Cellulase and Xylanase Production by *Micrococcus* species under Submerged Fermentation. *Sustainability*. 2016; 8:1168-1183.
24. Lowry OH, Roserbrough NH, Fann AL. Randall RJ. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951; 193: 265
25. Hongoh Y, Ekpornprasit, Inoue, T, Moriya S, Trakulnaleamsai S, Ohkuma M, Noparatnaraporn N, Kudo T. Intracolony variation of bacterial gut microbiota among castes and ages in the fungus-growing termite *Macrotermes gilvus*. *Mol. Ecol.* 2006; 15:505–516.
26. Shinzato N, Muramatsu M, Matsui T, Watanabe Y. Phylogenetic analysis of the gut bacterial microflora of the fungus-growing termite *Odontotermes formosanus* .*Biosci. Biotechnol. Biochem*. 2007; 71:906–915.
27. Zhu YY, Li J, Liu H, Yang H, Xin S, Zhao F, Zhang X, Tian Y, Lu X. Phylogenetic analysis of the gut bacterial microflora of the fungus-growing termite *Macroterme sbarneyi. Afr. J. Microbio. Res.* 2012; 6:2071-2078.
28. Sanders JG, Powell S, Kronauer, D. J. C., Vaconcelos, H. L., Frederickson, M. E. and Pierce, N. E. Stabiity and phylogenetic correlation in gut microbiota: lessons from ants and apes. *Mol. Ecol.* 2014; 23:1268-1283.
29. Liu N, Zhang L, Zhou H. Metagenomic insights into metabolic capacities of the gut microbiota in a fungus-cultivating termite (*Odontermes yunnanensis*). *PLosONE.* 2013; 8, e69184.
30. Dietrich C, Köhler T, Brune A. The cockroach origin of the termite gut microbiota: patterns in bacterial community structure reflect major evolutionary events. *Appl. Environ. Microbio*. 2014; 80(7):2261–69.

31. Kuhnigk T, Konig H. Degradation of Dimeric Lignin Model Compounds by Aerobic Bacteria Isolated from the Hindgut of of *Xylophagous termites*. *J. Basic Microbio.* 1997; 37:205-211.
32. Kuhnigk T, Borst E, Ritter A, Kampfer P, Graf A, Hertel H. Konig H. Degradation of Lignin monomers by the hindgut flora of termites. *Syst. Appl. Microbio.* 1994; 17:76-85.

33. Schafer A, Konrad R, Kuhnigk T, Kampfer P, Hertel H, Konig H. Hemicellulose –degrading Bacteria and Yeasts from the Termite Gut. *J. Appl. Bact.* 1996; 80:471-478.
34. Leadbetter JR, Schmid TM, Graber JR, Breznak JA. Acetogenesis from H_2 plus CO_2 by spirochetes from termite guts. *Science.* 1999; 283:686–689.
35. Lilburn TG, Kim KS, Ostrom NE. Nitrogen fixation by symbiotic and free-living spirochaetes *Science.* 2001; 292:2495-2498.
36. Graber JR, Leadbetter JR, Breznak JA. Description of *Treponema azotonutricium* sp. nov. and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. *Appl. Environ. Microbio.* 2004; 70:1315–1320.
37. Warnecke F, Hugenholtz P. Building on basic metagenomics with complementary technologies. *Gen. Biol.* 2007; 8:231.
38. Hongoh Y. Toward the Functional Analysis of Uncultivable, Symbiotic Microorganisms in the Termite Gut. *Cell. Mol. Life Sci.* 2011; 68(8):1311–1325.
39. Tanaka H, Aoyagi H, Shiina S, Doudou Y, Yoshimura T, Nakamura R, Uchiyama H. Influence of the diet components on the symbiotic microorganisms community in hindgut of *Coptotermes formosanus* Shiraki. *Appl Microbio Biotechnol*. 2006; 71:907–917.
40. Huang XF, Bakker MG, Judd TM, Reardon KK, Vivanco JM. Variations in diversity and richness of gut bacterial communities of termites (*Reticulitermes flaviceps*) fed with grassy and woody plant substrates. *Micro. Ecol.* 2013; 65:531-536.
41. Köhler T, Dietrich C, Scheffrahn RH, Brune A. High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (*Nasutitermes* spp.). *Appl. Environ. Microbio.* 2012; 78:4691–701.