ISOLATION AND CHARACTERIZATION OF BACTERIA ISOLATES FROM SOIL FEEDING TERMITES AND SOIL FROM JUJA AND KAKAMEGA FOREST IN KENYA

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Abstract
In the last several years information on the gut ecosystem of termites has continued to be gathered. Most studies have been focused on wood feeding termites but studies on soil feeders remain sparse owing to their difficulty of establishing permanent laboratory cultures. The aim of this study was to isolate, characterize and identify bacteria resident in the soil feeding termite gut, mound and parent soil of *Cubitermes* species with the potential to produce antibiotics and enzymes for industrialization. The samples were collected from kakamega forest from two sites Kalunya Glade and Lirhanda Hill. The study was also extended to the soils found in Juja. Hundred and thirty seven (137) isolates were cultivated and isolated using dilute nutrient agar media and screened for their antagonistic effects on various test organisms. Fifty one percent of the isolates were antagonist to *Escherichia coli*. Fifty seven percent of the isolates were antagonists to *Bacillus subtilis* while 55% of the isolates were antagonist to *Candida albicans*. Enzymatic activities of the isolates showed that 65% of the total isolates were starch degraders, 54% were casein degraders and 68% of the isolates were able to liquefy gelatin. Eleven percent of the isolates were cellulose degraders the majority of which were obtained from termite gut and mound. Isolates from Juja soil had the highest number of non degraders as compared to Kakamega Forest soils. The isolates were characterized using morphological, biochemical and molecular methods. Phylogenetic analysis of amplified 16S rRNA gene sequence revealed majority of the isolates were closely related to *Bacillus* and *Brachybacterium* species and had showed *invitro* antagonistic effects. Gram negative bacterial isolates obtained were closely related to *Pseudomonas* species. In conclusion, the isolates were potential antibiotic producers with varying ability to degrade gelatin, casein, and cellulose an indication of the role they play in their habitat.

Key words: DNBA, colony forming unit, mound, gut, surrounding soil, Kalunya, Lirhanda hill, Juja

1.0 Introduction

Termites are a ubiquitous feature of tropical and subtropical soils, where their number exceeds 6000 m^{-2} and their biomass densities (> 50 g m^{-2}) often surpass those of grazing mammalian herbivores (0.0-7.5 g m $^{-}$2) (Collins and Wood, 1984). They belong to the order Isoptera (Nutting, 1990). This order contains five families of lower termites and one family of higher termites (Noirot, 1992). Termites are recognized as one of the major ecosystems engineers in tropical soils (Holt and lepage, 2000). Their impart on soils is caused by their feeding habits where they play a vital role as mediators of decomposition (Wood and jonson, 1986), humification, soil conditioning, fragmentation of organic detritus, nitrogen- fixation, aggregate- binding and formation of clay- mineral complexes (Lee and Wood, 1971; Collins, 1983; Tayasu *et al*., 1994). Termites construct mounds from the mineral matrix with faeces or saliva depending on termite species (Grassie, 1984). These mounds increase the microbial density as a result of high organic matter (Brauman *et al.,* 2000). Diverse micro organisms inhabite the intestinal tracts of all termite feeding groups (Brune, 1998; Brauman *et al.,* 2001). Most studies on termite gut microbial communities have focused on wood feeding termites; analogous studies of other feeding guilds, especially soil feeders are lagging behind, owing to their typically remote habitat, delicate nature and the difficulty of establishing permanent laboratory cultures (Bignell *et al*., 1980; Rouland *et al*., 1993). Soil on the other hand is a highly heterogenous environment (Rolf, 2004), that contains a high diversity of microorganisms (Liesack *et al*., 2002). These microorganisms influence above ground ecosystems by contributing to soil structure and fertility among other roles (O'Dounell et al., 2001). Soil microorganisms are a valuable source of natural products providing important antibiotics for pharmaceuticals, enzymes and bioactive compounds for industries (Strohl, 2000). However the emergence of multidrug resistance pathogens has rekindled the need to discover new antimicrobials from remote environments.

Cultivation independent approaches based on molecular analysis have revealed unexplored bacterial diversity in soil. However such methods do not provide speculation on the microbial physiology, abundance and ecological significance hence the need for cultivation dependent approaches (Embley and Stackebrandt, 1996). Cultivation-dependent approaches have exploited the diversity of soil microorganism for many years based on the cultivation and isolation of microbial species (Rolf 2004). However microorganisms are endemic to certain geographic regions hence various habitats in Kenya may harbor unique microbes as a result of different soil composition and size of particles (Torsvik, 2002). Low nutrient media specifically dilute nutrient broth plus agar (DNBA) media have been used in the recent past to isolate rarely isolated groups of bacteria from soil and wood feeding termites at 25ºC incubation temperature (Janssen et al., 2002; Stevenson *et al*., 2004). This media compares to those present in the natural environments (Button *et al*., 1993) preventing the growth of fast growing bacteria a situation that is common with high nutrient media (Zengler *et al*.,). The aim of this study was to cultivate bacteria from soil, mound and soil feeding termite gut using low nutrient media and characterize them so as to identify potential antibiotic and enzyme producers.

2.0 Materials and Methods

2.1 Sampling

2.1.1 Kakamega Forest

Kakamega Forest is located in western part of Kenya and lies in the Lake Victoria basin 150kilometers west of the great African Rift Valley. It measures approximately 19,649 hectares and lies in altitude that ranges between 1500m and 1600m above sea level and up to 2060m for a few scattered forested hills such as Lirhanda where termites were collected. Kalunya Glade which is a marshy clay swampy region that lies between 800-100m above sea level and ranging in size from about 1 to 50 hectares consisting of grassy glade (Mutangah *et al*., 1992). The Forest receives 2080mm rainfall annually and has a temperature range of between 11^{o}C and 26^{o}C (Kakamega District Development Plan, 1994). The soils are generally Acrisols of low fertility medium to heavy textured clay loams and clay with Ph below 5.5 (FAO, 1989). Soil Feeding termites of *Cubitermes severus* species were collected from Kalunya Glade and Lirhanda hill in their respective mound and surrounding soil sampled one meter apart in three directions from the termite mound using a clean metal soil core with dimensions: 25 mm diameter and 100 mm depth.

2.1.2 Juja

Juja is located in lowland areas in the eastern parts of the Thika District. The District is located in the southern part of Central Province in the Kenya. It lies between latitudes 3° 35" and 1°45" south of the Equator and Longitudes 36º 35" and 37º 25" East (Thika District Development Plan, 2001). The climate of the area is generally semi arid and receives low rainfall of 856mm with a bimodal distribution. The area has an attitude of 1,060nm above sea level and mean annual temperature of 20ºC and the mean maximum temperature is 30ºC (Muchena *et al.*, 1978).

There are three types of soil in Juja, these are Shallow clay soils (Murram) coded ppm, deep clay soils (Vertisols) coded ppd and Soil associations and complexes coded ppc, **(**Muchena *et al*., 1978). The vegetation has *Acacia-Themeda* and common grasses such as *Themeda triandra* (Muchena *et al.,* 1978). Samples of soil were randomly collected from the three types of soil commonly found in the area. Soil cores were collected from the soil types using a clean metal soil core with the following dimensions: 25mm diameter and 100mm depth. The soil core was transported to the laboratory in sealed polyethylene bag at room temperature. The upper 30mm of each core are discarded and large roots and stones removed from the remainder that is then sieved through a sterile brass sieve of 2-mm aperture size.

2.2 Cultivation

Dilute nutrient broth plus Agar (DNBA) media used for cultivation was prepared with 0.08 g of nutrient broth solidified with 15 g Bacto agar (Difco) (Janssen *et al*., 2002) at a pH of 6.23. Ten termites (0.18 g) were degutted using sterile fine-tipped forceps. The gut sections were pooled and homogenized in 1ml basal salt solution (Leadbetter and Breznak, 1996 and Mackenzie *et al.,* 2007). A gram of freshly sieved soil/ mound was added to 9 ml aliquots of sterile distilled water and the solution was agitated using a vortex mixer at approximately 150 rpm for five minutes. Serial dilutions from the gut, soil and mound suspension were carried out up to 10^{-6}. Spread plates were then prepared from 0.1 ml of dilutions 10^{-4} and 10^{-5} (Janssen *et al*., 2002). The samples were also subjected to dilution and heat shock method where the dilutions were first preheated in a hot water bath (50 ^{o}C) for six minutes before spreading 0.1 ml of dilutions 10^{-4} and 10^{-5} on to DNBA plates (Mincer *et al.,* 2002). Cultivation was done in triplicate and all the 162 plates were incubated in the dark at temperatures 25 ^{o}C, 30 ^{o}C and 37 ^{o}C for 2 weeks (Janssen *et al*., 2002). Isolates were selected based on colony morphology and those exhibiting zones of inhibition on the primary culture. They were labeled based on sample and temperature at which they grew. These isolates were purified in dilute nutrient broth plus agar media and stocked at $^{-}$80ºC.

2.3 Morphological and Biochemical Characterization of Isolates

Morphological characterization was performed using gram stain and isolates were observed under inverted microscope at ×100 oil immersion (Cappuccino and Sherman, 2002). Biochemical tests on these isolates were performed as follows: Primary and secondary screening for antibiotic producing isolates against the test organisms *Bacillus subtilis* (NCIB3610*), Escherichia coli* (NCTC 10418) and *Candida albicans* (CACBS 562); Screening for enzyme activity using starch, gelatin, casein and cellulose as substrates; Indole production test; Hydrogen sulfide production test; motility; Nitrate reduction test; Urease test; Catalase test; Ability of isolates to grow in 7% sodium chloride. Ability of the isolates to utilize the following substrates: arabinose, maltose, fructose, sucrose, lactose, mannitol, xylose and xylan. This was determined using 2% of the substrate added to a solution that consisted of Nacl (0.1%), K_2HPO_4 (0.1%), $MgSO_4$ (0.05%), $FeSO_4.7H_2O$ (0.001%), $CuSO_4.7H_2O$ (0.0001%), $ZnSO_4.7H_2O$ (0.0001%g), $MnSO_4.7H_2O$ (0.0001%). Substrate utilization was determined after incubation by measuring turbidity that indicated growth. Uninoculated control for each substrate was used as a standard when measuring turbidity using Spectrophotometer at 560 nm (Cappuccino and Sherman, 2002; Harold, 2002).

2.4 Molecular Characterization

Identification of isolates was done through sequencing of 16S ribosomal RNA gene. DNA extraction was performed in 16 isolates using a method described by Sambrook *et al*., 1989. DNA purification was performed using QIAquick PCR purification Kit protocol (Qiagen, Germany). DNA amplification of the 16S ribosomal RNA genes was done using the QIAquick PCR purification kit (Qiagen, Germany) and bacterial primer pair 27F forward 5'-AGA GTT TGA TCC TGG CTC AG-3' in relation to Escherichia coli positions 8 to 27 (Edwards *et al*., 1989) and 1492R reverse, 5'-TAC GGY TAC CTT GTT ACG ACT T-3' Escherichia coli positions 1492 to 1512 (Wesburg *et al.,* 1991). The 16S rRNA gene sequences were compared using BLAST (http://www.ncbi.nih.gov) to those in the Genebank database

(Altschul *et al*., 1990; Shayne *et al*., 2003). The identification criteria used is as described by (Felsentein, 1989; Saitou and Nei, 1987).

3.0 Results

A total of one hundred and thirty seven pure isolates were obtained from fifteen samples that included soil, termite mound and termite gut. Screening for potential antibiotic production was performed as shown in Table 1 below.

Table 1: Sensitivity test of the active isolates on test organisms; B. subtilis E.coli, and C. albicans, using disc assay in cm

Sample	Isolate	*B.subtilis (NCIB3610)*	*E.coli (NCTC 10418)*	*C.albicans (CACBS 562)*
	Control	0.5	0.5	0.5
Juja soil asociation	84PPD30^{o}C	-	0.8	0.8c
Lirhanda B nest	29 LAN 30^{o}C	-	1.2	0.8
Lirhanda B Nest	57 LBN 30^{o}C	-	1.0	1.0
Lirhanda B nest	51 LBN 37^{o}C	-	1.0	1.1
Surrounding soil kalunya B	7 SKB 30^{o}C	0.9	1.0	1.1
Surrounding soil Lirhanda A	14 SLA 30^{o}C	0.8	0.8	-
Kalunya gut A	14 KAG 30^{o}C	0.8	-	-
Kalunya gut A	63KAG 25^{o}C	1.0	0.8	-
Lirhanda B Nest	24 LBN 30^{o}C	1.1	0.9	0.8
Surrounding soil Kalunya A	1 SKA 37^{o}C	0.8	0.8	0.8
Lirhanda gut B	62 LBG 37^{o}C	1.0	0.8	-
Murrum soil	142 M(b) 30^{o}C	1.0	0.7	-
Surrounding soil Lirhanda A	58 SLA 25 ^{o}C	1.0	1.0	-
Surrounding soil kalunya B	11 SKB 30^{o}C	1.0	1.0	0.9
Surrounding soil kalunya B	9 SKB 25^{o}C	1.0	-	1
Murrum soil	142 M 30^{o}C	-	-	-
Lirhanda gut B	1 1LBG 25^{o}C	0.7	-	0.7
Lirhanda B nest	LBN37^{o}C	0.8	0.7	0.8
Lirhanda gut B	LBG30^{o}C	0.7	-	0.7
Kalunya gut A	4KAG30^{o}C	0.7	-	0.7

Antimicrobial activity results for the 20 isolates (+) a positive result for the reaction and (-) a negative test for the reaction.

Ninety two (92) isolates were also screened for amylases, caseinases, gelatinases and cellulases. The percentage distribution of enzyme producing bacteria in the sample units was studied and summarized as follows: Sixty five percent (65%) of all isolates were starch degraders, Fifty four percent (54%) of the isolates degraded casein, sixty eight percent (68%) of the isolates degraded gelatin and Cellulose was degraded by 11% of the isolates obtained from termite gut. In addition 7% of isolates obtained from mound were cellulose degraders. The enzymatic activities of twenty (20) isolates whose antimicrobial activities were investigated in table 1 and characterized through biochemical tests (Table 3) are as shown in table 2 below.

Table2: Enzymatic activities of isolates

Isolate	Amylases	Caseinases	Gelatinases	Cellulases
84PPD30°C	+	-	+	-
29 LAN 30°C	+	-	+	-
57 LBN 30°C	-	+	+	-
51 LBN 37°C	-	+	+	-
7 SKB 30°C	+	-	+	-
14 SLA 30°C	+	+	+	-
14 KAG 30°C	+	-	+	-
63KAG 25°C	+	-	+	-
24 LBN 30°C	+	-	+	-
1 SKA 37°C	+	-	+	-
62 LBG 37°C	+	-	+	-
142 M(b) 30°C	+	-	+	-
58 SLA 25°C	+	+	+	-
11 SKB 30°C	+	+	-	+
9 SKB 25°C	-	+	+	-
142 M 30 °C	+	+	+	-
1 1LBG 25°C	-	+	+	-
2LBN37°C	+	+	+	+
3LBG30°C	+	+	+	+
4KAG30°C	+	+	+	-

Enzymatic activity results for the 20 isolates (+) a positive result for the reaction and (-) a negative result for the reaction

Table 3: showing biochemical test of 20 isolates that were under investigation

ISOLATE	NIT	URE	XYL	IND	MAN	ARA	MAL	FRU	SUC	LAC	NCL7%	XY
24LBN30°C	+	-	nd	-	nd	+	+	+	+	-	nd	nd
1 SKA37 °C	+	-	nd	-	nd	+	-	+	+	-	nd	nd
62LBG37°C	+	-	nd	-	nd	+	-	+	+	-	nd	nd
63KAG37°C	+	-	nd	-	nd	+	+	+	+	-	nd	nd
58SLA25°C	+	-	nd	-	nd	+	+	+	+	-	nd	nd
14KAG30 °C	+	-	nd	-	nd	+	-	+	+	-	nd	nd
14SLA30 °C	+	-	nd	-	nd	+	-	+	+	-	nd	nd
142MB30 °C	+	-	nd	-	nd	+	-	+	+	-	nd	nd
142M30 °C	+	+	nd	+	nd	-	-	-	-	+	nd	nd
51LBN37 °C	+	+	nd	+	nd	-	+	+	-	-	nd	nd
57LAN30 °C	+	+	nd	+	nd	-	+	+	-	-	nd	nd
29LAN30 °C	+	+	nd	+	nd	-	+	+	-	-	nd	nd
11SKB30 °C	+	-	+	-	+	-	+	+	+	+	+	nd
84PPD30 °C	-	-	nd	+	nd	-	+	+	+	-	nd	nd
7SKB30 °C	-	-	nd	+	nd	+	+	+	+	-	nd	nd
9SKB 25 °C	+	+	nd	-	nd	-	-	+	+	+	nd	nd
1LBG25 °C	-	-	+	-	+	nd	nd	nd	+	nd	+	-
2LBN37 °C	-	+	-	-	-	nd	nd	nd	-	nd	+	-
3KAG 0 °C	+	+	+	-	-	nd	nd	nd	-	nd	+	-
4LBG30 °C	+	+	+	-	+	nd	nd	nd	+	nd	+	+

Biochemical test result for 20 isolates (+) positive reaction,(-) negative reaction and (nd) not determined.NIT-nitrates,URE-ureas,XYL-xylose,IND-indole,MAN-mannitol,ARA-D arabinose,MAL-maltose,FRU-fructose,SUC-sucrose,LAC-lactose,NCL7%-sodium chloride,XY-xylan

3.1 Morphological Characterization of Isolates

Morphological studies of the isolates was done using the dissecting (×16) and light microscopes (×100 oil immersion). Majority of the isolates obtained were rod shaped gram positive bacteria as shown in Table 4.

Table 4: Morphological characteristics of isolates

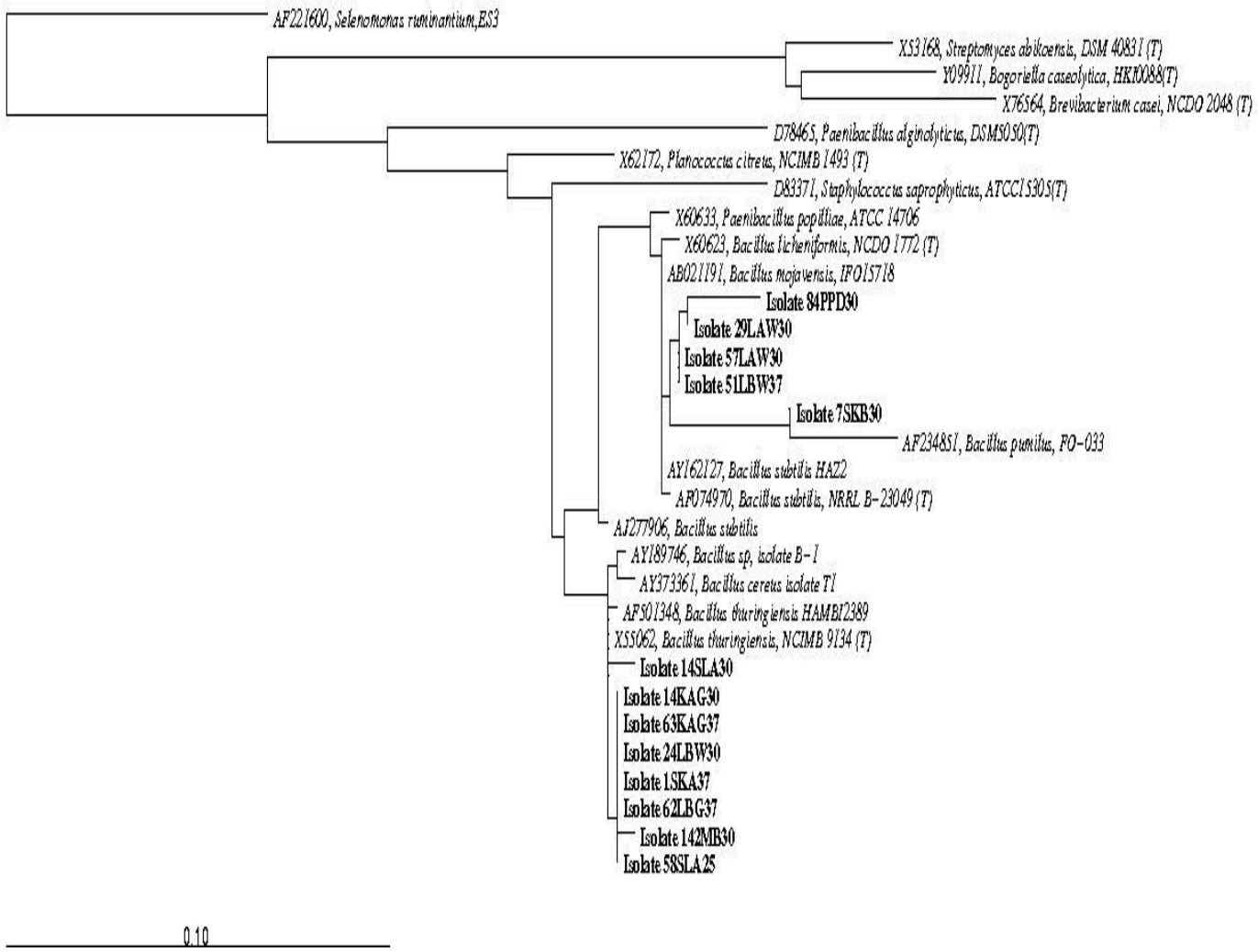
Isolate	Gram	Cell shape	endospore	Colony morph.	color	Pigment	Motility
24 LBN30 ^{o}C	+	Rod	+	Circular ,slightly raised , entire	Cream white	-	+
1 SKA 37 ^{o}C	+	Rod	+	Circular ,slightly raised , entire	Cream white	-	+
62 LBG37 ^{o}C	+	Rod	+	Circular, slightly raised, entire	Cream white	-	+
63KAG 37 ^{o}C	+	Rod	+	Circular ,slightly raised ,entire	Cream white	-	+
58SLA25 ^{o}C	+	Rod	+	Circular ,slightly raised ,entire	Cream white	-	+
14KAG30 ^{o}C	+	Rod	+	Circular ,slightly raised entire	Cream white	-	+
14SLA30 ^{o}C	+	Rod	+	Circular ,slightly raised ,entire	Cream white	-	+
142MB30 ^{o}C	+	Rod	+	Circular ,slightly raised , entire	Cream white	-	+
142M30 ^{o}C	-	Rod	-	Circular, serrate flat	Cream white	Green	+
51LBN37 ^{o}C	+	Rod	+	Circular, mucoid. convex,	Cream white	-	+
57LAN30 ^{o}C	+	Rod	+	Circular, mucoid. convex,	Cream white	-	+
29LAN30 ^{o}C	+	Rod	+	Circular, mucoid. convex,	Cream white	-	+
11SKB30 ^{o}C	+	short rod	-	Circular,entire,convex	Cream white	-	-
84PPD30 ^{o}C	+	Rod	+	Circular, mucoid. convex,	cream white	-	+
7SKB30 ^{o}C	+	Rod	+	Circular entire, raised	Cream	-	+
9SKB 25 ^{o}C	-	Coccid rod	-	Circular, entire slightly raised	pink	pink	+

Morphological test for the 16 isolates (+) a positive result or presence and (-) a negative result /no presence. Colony morph.-colony morphology

3.2 Phylogenetic Cluster Analysis of Sequences

The 16SrRNA gene amplified products from sixteen isolates (Table 7) were successfully sequenced. Phylogenic analysis using BLAST software (http://www.ncbi.nih.gov) showed that they belong to the domain Bacteria and phyla *Firmicutes, Proteobacteria and Actinobacteria.* Thirteen isolates were clustered with the low G+C content *Firmicutes* in to three clusters: Isolates 1SKA37^{o}C, 58SLA25^{o}C, 142MB30^{o}C, 14SLA30^{o}C 14KAG30^{o}C, 62LBG37^{o}C, 63KAG37^{o}C and 24LBN30^{o}C all clustered with *Bacillus thuringiensis*. It was supported with a sequence similarity of 99.3%-99.7% and bootstrap value of 99%. On the other hand isolates 51LBN37^{o}C and 57LBN37^{o}C from termite nest were clustered with *Bacillus mojavensis* with a sequence similarity of 99.7% and bootstrap value of 99%. Isolate 29LAN 30 ^{o}C and 84PPD30 ^{o}C were clustered with *Bacillus subtilis* and respectively with a sequence similarity of 99.5% and bootstrap value of 99%. Lastly, isolate 7SKB 30 ^{o}C from soil was clustered with *Bacillus pumilus* with a sequence similarity of 99.33% and bootstrap value of 99% as shown in Figure 1 below.

Figure 1: Phylogenetic tree showing positions of isolates closely related to *Firmicutes*. The scale bar indicates approximately 10% sequence difference. Included are sequences from termite gut, mound and soil. 1SKA37°C, 58SLA25°C142MB30°C, 14SLA30°C, 14KAG30°C, 62LBG37°C), 63KAG37°C, 24LBN30°C, 51LBN37°C57LBN37°C, 29LAN30°C, 84PPD30°C, 7SKB30°C



The second phylum that was represented in the phylogenetic analysis was the *Actinobacteria* phylum. Isolate 11SKB 30^{o}C was clustered with *Brachybacterium paraconglomeratum* (Figure 2) with a sequence similarity of 100% and bootstrap value of 99% in the Genbank database.

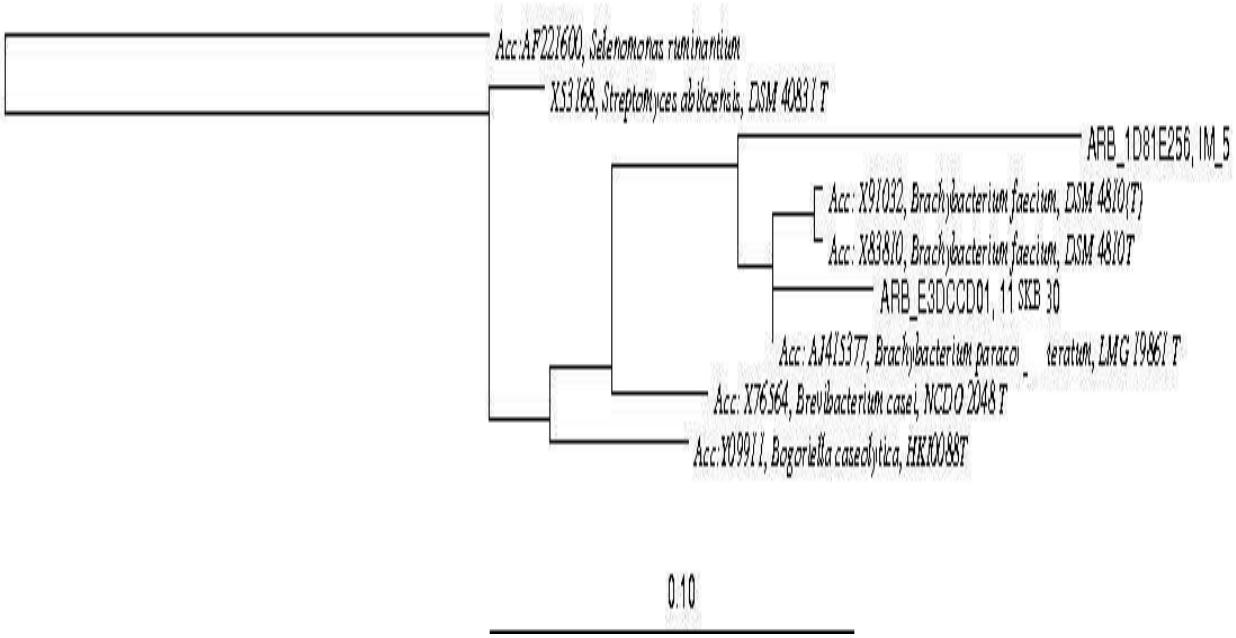


Figure 2: Phylogenetic tree showing positions of isolates closely related to Actinobacteria phyla. The scale bar indicates approximately 10% sequence difference. Included are sequences from isolate 11SKB 30^{o}C

The third phylum represented in the phylogenetic analysis of isolates was the *Proteobacteria* phylum. Isolate 142M30^{o}C was clustered with *Pseudomonas aeruginosa* (Figure 3) with a sequence similarity of 99% and bootstrap value of 98% in the Genbank database. The phylum *Proteobacteria* phylum was also represented by isolate 9SKB 25^{o}C clustered with *Serratia marcescens* (Figure 3) with a sequence similarity of 99.4% and bootstrap value of 98%. The species *Serratia marcescens* belongs to the family Enterobacteriacea.

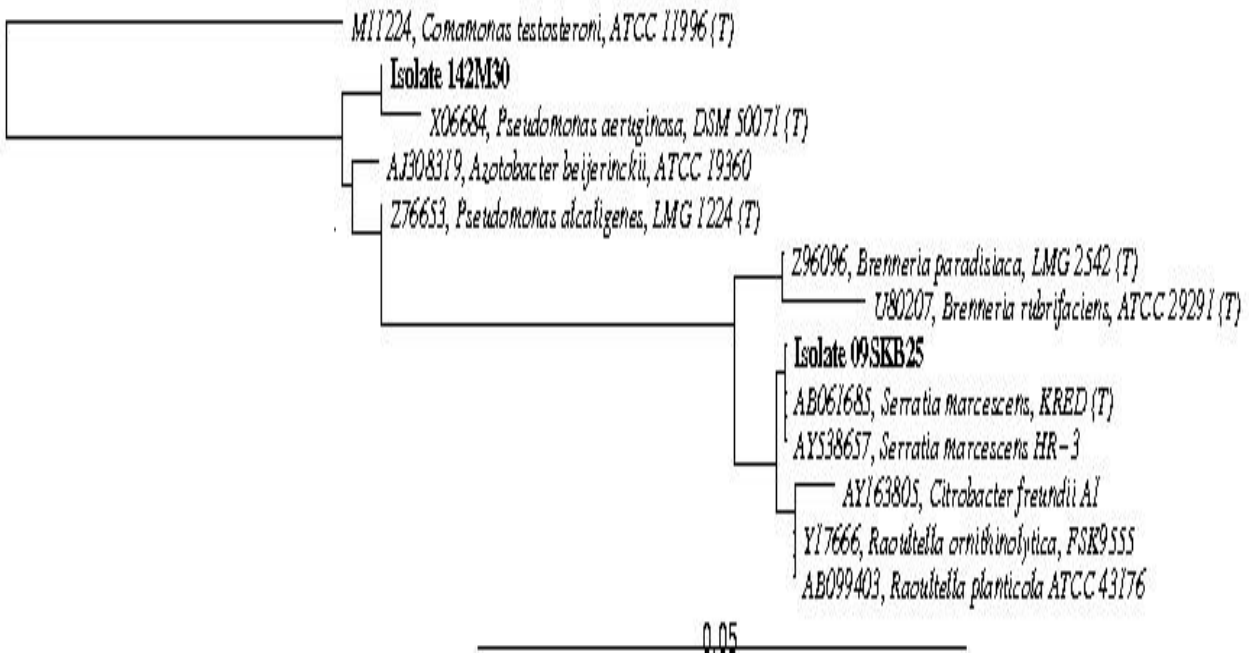


Figure 3: Phylogenetic tree showing positions of isolates closely related to proteobacteria phyla. The scale bar indicates approximately 50% sequence difference. Included are sequences from soil isolates 142M30^{o}C and 9SKB 25^{o}C

4.0 Discussion

The media (DNBA) used in this study was favorable as it allowed the development of a wide range of bacteria present in the sample, prevented growth of spreading colonies and allowed the least possible growth of unwanted non-bacterial colonies such as fungi (Janssen *et al*., 2002).

The study showed majority of isolates were gram positive bacteria and few gram negative (Table 4). Previous studies had shown that enteric bacteria have difficulty competing with natural micro flora for the low concentration of available nutrients in natural ecosystems (Burton *et al.,* 1987). Instead the enteric bacteria have grown with much ease in most standard nutrient rich laboratory media preventing growth of the majority micro flora found in natural ecosystems. In this study there were few enteric bacteria (Figure 3) as a result of low nutrient media used.

Prokaryotic microorganisms have been previously shown to inhabit the gut of soil feeding termites (Brune, 1998; Brauman *et al*., 2000). Previous analysis of soil feeding termite gut using PCR- denaturing gradient gel electrophoresis (DGGE) revealed presence of *Firmicutes* phyla in the termite gut library (Fall *et al.,* 2007). Morphological characterisation in this study showed majority of termite gut isolates were Gram positive-rod shaped, endospore forming and motile bacteria (Table 4). Phylogenetic analysis showed that these isolates were closely related to members in the bacillus group (Figure 2). Through biochemical characterisation (Table 3), these isolates showed ability to reduce nitrates to nitrites and nitrogen supporting the hypothesis that nitrogenous components of humus are an important dietary resource for humivorous soil macro invertebrates (Ji *et al.,* 2000; Ji and Brune, 2001).

Phylogenetic analysis of isolates also revealed members closely related to the bacilli group in the termite mound and surrounding soil (Figure 1). Previous analysis of soil feeding termite mound using PCR- denaturing gradient gel electrophoresis (DGGE) revealed presence of *Firmicutes* phyla in the termite mound and surrounding soil library but to a lesser extent when compared to those found in the gut samples (Fall *et al.,* 2007). Biochemical characterisation showed that these isolates (Bacilli group) were nitrate and nitrites reducers (Table 3) indicating the role these isolates play in the nitrogen cycle of termite mound, gut and soil (Collins, 1983**).**

Previous studies have shown presence of isolates able to utilize glucose (Boga *et al*., 2007). In this study the isolates were able to utilize arabinose, maltose, sucrose and fructose (Table 3) an indication of the role played by these isolates in the formation of short chain fatty acids from carbohydrates or synthesis of amino acids (Cummings and Macfarlane, 1997).

Isolates whose sequences were closely related to *Serratia marcescens* and *Pseudomonas aeruginosa* were obtained from surrounding soil and Juja soil samples respectively (Figure 3). Morphological characterisation revealed the isolates were Gram negative, rod-shaped and motile (Table 4) bacteria belonging to *Proteobacteria,* phylum (Bergy's manual, 1989). Previous analysis of soil feeding termite surrounding soil using PCR- denaturing gradient gel electrophoresis (DGGE) revealed the presence of *Proteobacteria* phylum in the surrounding soil library (Fall *et al.,* 2007) hence this study was able to cultivate them in low nutrient media. Biochemical characterisation of the isolate closely related to *Serratia marcescens* revealed ability to utilize fructose, sucrose and lactose (Table 3). These isolates were also able to reduce nitrate (Table 3) indicating the role it could play in the nitrogen cycle (Lengeler *et al*., 1999) in its habitat.

Phylogenetic analysis further revealed an isolate closely related to *Brachybacterium paraconglomeratum* (Figure 3) obtained in the surrounding soils of termite gut. Previous analysis of soil feeding termite surrounding soil using PCR- denaturing gradient gel electrophoresis (DGGE) revealed presence of members of Dermabacteraceae family in the nearly half of the surrounding soil clone library (Fall *et al*., 2007). This *Brachybacterium* species belongs to the Dermabacteraceae family and *Actinobacteria* phylum (Stackebrandt, 1997). It is a Gram positive, coccid-rod shaped, non spore forming non motile (Table 4) and high G+C content bacteria (Takeuchi *et al.,* 1995). The ability of this isolate to reduce nitrate indicates the role it plays in the nitrogen cycle (Lengeler *et al*., 1999). Substrate utilisation tests of the isolate revealed the ability to utilize lactose, xylose, mannose, fructose, galactose, maltose and sucrose (Takeuchi *et al.,* 1995).

Potential antibiotic producers in termite gut and mound samples were clearly shown in this study (Table 1). This is consistent with previous studies that showed the *Bacillus* species are capable of producing antibiotic as secondary metabolites (Katz and Demau, 1977). Isolate 11SKB 30^{o}C obtained from surrounding soil that was closely related to *Brachybacterium* species was capable of producing antagonistic effects against the test organisms (Table 1). *Brachybacterium* species has been used in previous studies to eliminate *Staphylococcus aureus* (Takeuchi *et al.,* 1995)

Members of genus *Bacillus* are capable of secreting a wide variety of enzymes such as amylases and proteases (Priest 1977; Mezes and Lampen, 1985). In this study, isolates obtained from termite gut closely related to *Bacillus* species had the ability to degrade starch, an indication of the role the amylases play in extracting organic matter from the soil in the gut, a process that is favoured by alkaline conditions in the gut (Brune, 1998; Kappler and Brune, 1999). The termite gut isolates capable of degrading gelatin (Table 2) is in line with previous studies that have shown presence of aerobic organisms in the gut capable of degrading gelatin (Boga *et al*.,2007). In addition isolates in termite mound and surrounding soil respectively were able to degrade gelatin (Table 2). Gelatin degrading isolates (Table 2) were closely related to members of Genus *Bacillus* (Figure 1) in line with previous investigations by Debabor (1982). Isolate 11SKB 30^{o}C thats closely related to *Brachybacterium* species (Figure 2) was aslo able to degrade gelatin in line with previuos investigations by Takeuchi *et al.,* (1995) an indication of the role they play in transformation of peptides and protein in their habitat.

Brune and his co-workers were able to show that soil-feeding termites transform cellulose in the soil an indication of presence of cellulolytic bacteria in the gut (Ji and Brune, 2001). In this study, there were termite gut and mound

isolates capable of degrading cellulose indicating the role played by these bacterial isolates in degrading cellulotic materials in termite mound (Table 2). The Ability of the majority of isolates from soil, gut and mound to utilize various sugars (Table 3) is an indication of the role they play in generation of short chain fatty acids from carbohydrates (Cummings and Macfarlane, 1997).

5.0 Conclusions

The study has demonstrated using low nutrient media (DNBA) in cultivation, the isolation and characterization of bacterial isolates in the termite gut, mound and soil and further shown that these samples are a potential source of antibiotic and enzyme producing bacteria.

6.0 Recommendation

To get a clear insight into the mechanism of antagonism, further studies should be done to characterize and identify the proteins or compounds with antimicrobial activity.

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References

Altschul, S. F., Gish, W. Miller, E. W. Myers and lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology,* **215**, pp 403-410.

Atlas, R. M., (1984). Diversity of Microbial Communities volume 7 New York Plenum Press.

Bergy's manual, (1989) of systematic bacteriology, volume 4, eds. S.T.Williams and M.E.Sharpe, Baltimore, Md: Williams and Williams.

Bignell, D. E., H. Oskarsson and J. M. Anderson, (1980). Distribution and abundance of bacteria in the gut of a soil–feeding termite Procubitermes aburiensis (Termitidae, Termitinae). *Journal of General Microbiology,* **117**, Pp 393–403.

Boga, H. I., Ji, Ludwig, R. W. and Brune, A. (2007). *Sporotaela propionica* genera novel species. Hydrogen oxidizing, Oxygen reducing, propiogenic firmicutes from the intestinal tract of a soil feeding termite. *Archives of Microbiology,* **187** (1), pp 15-27.

Brauman, A., Bignell, D. E. and Tayasu, I. (2000). Soil–feeding termites: biology, microbial associations and digestive mechanisms *In:* T. Abe, D. E. Bignell, and M. Higashi (eds.) Termites: evolution, sociality, symbiosis, ecology. Kluwer Academic Publishers, Dordrecht. Pp 233–259

Brauman, A., Dore, J., Eggleton, P., Bignell, D., Breznak, J. A. and Kane, M. D. (2001). Molecular phylogenetic profiling of prokaryotic communities in guts of termites with different feeding habits. *FEMS Microbiology Ecology,* **35**, pp 27–36.

Brune, A., (1998). Termite guts: the world's smallest bioreactors. *Trends in Biotechnology*, **16**. Pp 16–21.

Burton, G.A., D. Gumison, and G. R. Lanza, (1987). Survival of pathogenic bacteria in various fresh water sediments. *Applied and Environmental Microbiology* **53**, pp 633-638.

Button, D. K., Schunt, F., Quang, P., Martin, R. and Robertson, B. R. (1993). Viability and isolation of typical marine oligotrophic bacteria by dilution culture theory, procedures and initial results. *Applied and Environmental Microbiology*, **59**, pp 881-891.

Cappuccino, J. G. and Sherman, N. (2002). Microbiology. A laboratory manual. 6th edition. Pearson education inc. San Francisco, California, pp 215-224

Claus, D. and Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872, (2)1105-1139. **In**: P.H.A. Sneath, (eds.), Bergey's Manual of Systematic Bacteriology, Volume 2. Williams and Wilkins Co., Baltimore, MD.

Collins, N. M., (1983). The utilization of nitrogen resources by termites (Isoptera) **In**: J. A. Lee, S. McNeill, and I. C. H. Rorison (eds.) Nitrogen as an Ecological Factor. Blackwell Scientific Publications, Oxford, pp 381–412.

Collins, N. M. and T. G. Wood, (1984). Termites and atmospheric gas production. *Science* **224**, pp 84–86.

Cummings, J. H. and MacFarlane, G. H. (1997). Role of intestinal bacteria in nutrient metabolism. *J Parenter Enteral Nutr.,* **21**, pp 357-365.

Debabor, V. G., (1982). The industrial use of bacilli 331-370. D.A. Dubnau (eds). The molecular biology of the bacilli. Academic press .New York.

Embley, M. T. and E. Stackebrandt, (1996). Molecular Approaches to Environmental microbiology (Editors. pickup, R.w. and Saunders J.R.), Prentice Hall, United Kingdom, pp 39-62.

Fall, S., Hawelin, J. Ndiaye, F., Assigbetse, K., Aragno, M. Chotte J. L. and Brauman, A. (2007). Differences between Bacterial Communities in the gut of a soil feeding termite (*Cubitermes niokoloensis*) and its mound. *Applied and Environmental Microbiology,* **73**(16), pp 5199-5208.

FAO(Soil reference) 1989 Food and Food security .FAO Forestry paper, FAO (UN), **90**, pp1-2.

Felsentein, J., (1989). PHYLIP--phylogeny interference package (version 3.2). *Cladistics,* **5**, pp 164-166.

Government of Kenya, (1994). Kakamega District Development Plan 1994-1996. Office of the Vice President and Ministry of Planning and National Development.

Government of Kenya, 1997. Thika District Development Plan 1997-2001 Office of the Vice President and Ministry of Planning and National Development.

Grass'e, P. P., (1984). Terminology Foundation Dessocietes-Construction Volume 2 Masson Paris France.

Holt, J. A. and Lepage, M. (2000). Termites and soil properties 389-407. In T. Abe. D.E Bignell and M. Higashi (eds), Termites evolution, sociality, symbioses, ecology, Volume 1 Kluwer Academic, Dordrecht. Netherlands.

Janssen, P. H., Yates, P. S., Grinton, E. B., Taylor, P.M. and Sait, M. (2002). Improved culturability of soil Bacteria and isolation in Pure culture of novel members of the division Acidobacteria, Actinobacteria, Proteobacteria and Verrucomicrobia. *Applied and Environmental Microbiology*, **68**, pp 2391-2396.

Ji R., A. Kappler and A. Brune, (2000). Transformation and mineralization of synthetic ^{14}C–labeled humic model compounds by soil–feeding termites. *Soil Biology and Biochemistry,* **32,** pp 1281– 1291.

Ji, R., and A. Brune, (2001). Transformation and mineralization of 14 C-labeled cellulose, peptidoglycan. *Soil Biology and Biochemistry,* **32**, pp 1281–1291.

Kappler, A. and Brune, A. (1999). Influence of gut alkalinity and oxygen status onmobilization and size–class distribution of humic acids in the hindgut of soil–feeding termites. *Applied and Soil Ecology*, **13**, pp 219–229.

Katz, E. A. and L. Demari, (1977). Tripeptide antibiotics of Bacillus; Chemistry, Biogenesis and possible functions Bacterial Review, **41**, pp 449-474.

Leadbetter, J. R. and J. A. Breznak, (1996). Physiological ecology of *Methanobrevibacter cuticularis* species novel and *Methanobrevibacter curvatus* species novel isolated from the hindgut of the termite *Reticulitermes flavipes*. *Applied and Environmental Microbiology,* **62**, pp 3620-3631.

Lee, K. E. and T. G. Wood, (1971). Termites and Soils, Academic Press New York.
Lengeler, J. W., G Drews and H.G. Schlegel. 1999. Biology of the Prokaryotes. Blackwell Scientific. Malden.MA.

Liesack, W., Janssen, P. H., Rainey, F. A., Ward-Rainey, N. L. and Stackebrandt, E. (1997). Microbial diversity in soil: The need for a combined approach using molecular and cultivation Techniques. In modern soil Microbiology. Elsas, J.D., Trevors, J. T. and Wellington E. M. H (Editors) New York: Marcel Dekker, pp 375-439.

Mackenzie, L. M., A. T. Muigai, E. O. Osir, W. Lwande, M. Keller, G. Toledo and H. I. Boga, (2007). Bacterial Diversity in the intestinal tract of the Fungus Cultivating termite *Macrotermes michaelseni. African Journal of Biotechnology*, **6**(6), pp 658-667.

Mezes, P. S. and J. O. Lampen, (1985). Secretion of proteins by Bacillus 151-185. D.A. Dubnau (ed). The molecular biology of the bacillus, Volume 2. Academic Press,New York.

Mincer, T. J., P. R. Jensen, C. A. Kauffman and W. Fanical 2002. Widespread and persistent populations of a major new marine Actinomycete taxon in ocean sediments. *Applied and Environmental Microbiology,* **68**, pp 5005–5011.

Muchena, F. N., W. M. Wamich, C. R. K. Njoroge, (1978). Detailed Soil Survey of Jomo Kenyatta University of Agriculture and Technology (Juja) (Kiambu District) Ministry of Agriculture-National Agricultural Laboratories Nairobi 1-25.

Mutangah, J. G., O. M. Mwangangi and P. K. Mwaure, (1992). Kakamega Forest Vegetation survey. Kenya indigenous Forest Conservation Program National Museum of Kenya 248-259.

Noirot, C., (1992). From wood– to humus–feeding: an important trend in termite evolution. In: Biology and Evolution of Social Insects (Billen, J., ed) 107–119. Leuven University Press, Leuven, Belgium.

Nutting, W. L., (1990). Insecta Isoptera .**In**: Dindal, D. L. (Eds) Soil Biology Guide. John Wiley and Sons, New York 997-1032.

O'Donnnell, A. G., M. Seasman, A. N. Macne, T. Davies, (2001). Plants and fertilizers as drivers of change in Microbial Community Structure and Function in soil. *Plant soil,* **232**, pp 135-145.

Priest, F. G., (1977). Extra cellular enzymes synthesis in the genus *Bacillus* Bacterial. Review **41**, pp 711-753.

Rolf, D., (2004). The soil metagenome as a rich resource for the discovery of natural products. *Current Opinion in Biotechnology,* **15**. Pp 199-204

Rouland, C., A. Brauman, M. Labat and M. Lepage, (1993). Nutritional factors affecting methane emission from termites. Chemosphere **26**, pp 617–622.

Saitou, N. and M. Nei, (1987). The neighbor-joining method. A new method for reconstructing phylogenetic trees. Molecular Biology. *Evolution*, **4**, pp 406-425.

Sambrook, J., E. F. Fritsch and T. Maniatis, (1989). Molecular cloning: a laboratory manual, 2nd edition Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schmitt–Wagner, D., M. W. Friedrich, B. Wagner and A. Brune (2003a). Phylogenetic diversity, abundance, and axial distribution of bacteria in the intestinal tract of two soil–feeding termites *(Cubitermes* species). *Applied and Environmental Microbiology,* **69**, pp 6007–6017.

Shayne, J. J., P. Hugenholtz, P. Sangwan, C. Osborne and H. P. Janssen, (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. *Applied and Environmental microbiology*, **69**, pp 7211-7214.

Stackebrandt, E., F.A. Rainey and N.L. Ward-Rainey, (1997). Proposal for new Hierarchic Classification System, Actinobacteria classics Novel*. International Journal for Systemic Bacteriology,* **47**, pp 479-491.

Stevenson, B. S., A. Stephanie, J. Fichorst, T. Wertz, T. M. Schmidt and J. A. Breznak, (2004). New strategies for cultivation and detection of previously uncultured microbes. *Applied and Environmental Microbiology,* **70**(8), pp 4748-4755.

Strohl, W. R., (2000). The Role of natural products in modern drug discovery. *Drug Discovery Today* **5**, pp 39-41.

Takeuchi, M. and Fang, C. X. (1995). Taxonomic study of the genus *Brachybacterium*: proposal of *Brachybacterium conglomeratum* sp. Nov., nom. rev., *Brachybacterium paraconglomeratum* sp. Nov., and *Brachybacterium rhamnosum* sp. nov. *International Journal of Systemic Bacteriology*, **45**, pp 160-168.

Talaro, A. and K. Talaro, (1996). Drugs microbe Host. The elements of Chemotherapy in foundations in Microbiology W.M., Brown: New York.

Tayasu, I., A. Sugimoto, E. Wada and T. Abe, 1994. Xylophagous termites depending on atmospheric nitrogen. *Naturwissenschaften,* **81**, pp 229–231.

Thomas, S. B. and E. Lewis, (1957). Some Biochemical Characteristics of Freshly isolated strains of the Genus Serratia. *Journal of Applied Bacteriology,* **20**(1), pp 11-16.

Torsvik V., F.L Daa, R.A. Sandaa and L. Orneas, 2002. Microbial diversity and function in soil: From genes to Ecosystems. *Current Opinion in Microbiology*, **5**, pp 240-245.

Weisburg, W.G., S. M. Barns, D. A. Pelletier and D.J. Lane, 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, **173**, pp 697-703.

Wood, T. G. and R. A. Johnson, (1986). The biology, physiology, and ecology of termites In: Economic impact and control of social insects (Vinson, S. B., Ed.). 1–68. Praeger, New York.

Zengler, K., G. Toledo, M. Rappé, J. Elkins, E. J., Mathur, J. M. Short and M. Keller,(2002). Cultivating the uncultured. National academy of science USA, 99:15681- 115686.