



# Isolation, Identification and Screening of Bacteria with Antibiotic Production Potential from Termite Mounds

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**Abstract:** Antibiotics are of great importance in medicine, that are harmful to the growth and metabolic activities of bacteria. Ever increasing search is going on in the hope that agents' superior to these and other antibiotics now in use might be isolated. Present study seeks to screen bacteria with antibiotic producing potentials from termite mounds. Eight (8) termite mound samples were collected from the study sites (Shamawa, Dundaye, G/Yaro and Gumburawa), processed and bacteria were isolated and identified using culturing methods, gram staining and biochemical characterization methods. The isolates were further tested for antibiotic production using crowded plate techniques on muellar-hilton agar. Morphological and cultural studies shows that twenty gram positive and six gram negative bacteria species were identified namely *Bacillus* spp, *Citrobacter freundii*, *Enterococcus cloacae*, *Enterococcus faecalis*, *E. coli*, *K-pneumoniae*, *Pseudomonas* spp, *Staph aureus*, *Staph lentus* and *Staph ureae*. Eleven bacteria species out of the fourteen isolates showed antibiotic production activity against *Staphylococcus epidermidis* and 3 were not active against the test organism. These include *E. coli* and *Pseudomonas* spp. The zone of inhibition formed are *Bacillus cereus* 4 mm, *Bacillus subtilis* 5 mm, *Bacillus megaterium* 2 mm, *Citrobacter freundii* 4 mm, *Enterococcus cloacae* 2 mm, and *Enterococcus faecalis* 2 mm respectively. The result of this research indicates that termites mound may be used as a source of antibiotic producing bacteria.

**Keywords:** Bacillus Spp, Pseudomonas Spp, Staphylococcus Spp, Enterococcus Spp

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## 1. Introduction

Antibiotics are substances that are produced by microorganisms and are harmful at low concentrations to the growth and metabolic activities of other organisms. Powerful antibiotics such as penicillin and cephalosporin are of such tremendous importance in medicine, that an ever-increasing search is going on in the hope that agent superior to these and other antibiotics now in use might be isolated [4].

According to [3], higher forms of life such as algae, lichens, plants and animals have been shown to produce anti-microbial substances of low molecular weight. These are secondary metabolites, just like conventional antibiotics. Because of this, over the past two decades, there has been a tendency to extend the term antibiotic to all secondary metabolites, irrespective of

their origin, which are able to inhibit various growth processes at low concentration [13] and [4]. It is not altogether an unreasonable redefining of terms, since the word antibiotic, derives from two origins, anti (against) and bios (life) [14]. Nothing in the word itself, either in origin or in use, restricts the term antibiotic to substances of microbial origin. Proponents of this view argue that any products from a living organism which, at low concentrations, kill any other living organism, be they microorganisms, higher plants or animals, should be described as antibiotics [9].

## 2. Materials and Methods

### 2.1. Collection of the Mound Samples

One hundred (100 g) of termite mound materials was

collected from eight (8) different location in the study area in new clean polythene bags. Authentication of mounds material was done in the Soil Science laboratory Department of Soil science and Agric Engineering Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Nigeria.

## 2.2. Preparation of Mound Materials

Mound samples were processed separately as described by [1], each mound material was broken and pounded with the aid of mortar and pestle to a coarse powder and this was sieved with a 2 mm sieve to remove contaminants and to obtain a composite sample. The powdered samples was stored in black polythene bags and stored in clean sterile container for further analysis [13] and [11].

## 3. Microbiological Analysis

### 3.1. Isolation of Antibiotic Producing Bacteria

Eight (8) termite samples were collected from the study site (Shamawa, Gumburawa, Dundaye and G/yaro) separately at 45°C for 1 h in a hot air oven and then cooled to room temperature. The soil sample (1 g) was added to a conical flask containing 100 ml of sterile water. The flasks were shaken for 30 min in an orbital shaker incubator at 37°C and their contents designated stock cultures [3].

Screening of mound samples by crowded plate technique.

A series of culture tubes containing 9 ml of sterile water was taken. From the stock culture, 1 ml suspension was transferred aseptically to the 1st tube (101) and mixed well. Further serial dilutions were made to produce 10<sup>-6</sup> suspensions were made.

Suspension (0.1 ml) from each culture tube was spread on sterile soya medium plates, and nutrient agar plates aseptically in a laminar-air flow cabinet [6]. The plates were incubated at 37°C for 24 h. The plates were observed intermittently during incubation [5].

After 24 hrs, whitish pink colonies, characteristic of bacteria and with a clear zone of inhibition around them were seen.

The whitish pink colonies with inhibitory or clear zone of inhibition were selected and purified into a Trypticase agar slants. The selected strains were further purified by multiple streaking method [12]. The stock cultures of each selected strain was prepared and maintained in Trypticase agar slants at +4°C. The bacterial colonies isolated from the crowded plate were selected for the further studies.

### 3.2. Preliminary Screening of Crude Antibiotic AGAR Streak Method

The bacterial sensitivity of the mound isolates was analyzed by agar streak method [8]. Each of the isolate was streaked as a straight line on Trypticase medium and incubated at 37°C for 6 days (144 h), [2] and [15]. After the 6th day, different strains of bacteria were streaked at right angle, but not touching each other, and then incubated at 37°C for 24 h in the case of bacteria [10]. If the organism is susceptible to the antibiotic produced by bacteria, then it will not grow near the isolates. The zone of inhibition against each test organism was noted. The isolated bacteria were screened against some microorganisms (*S. epidermidis*). Based on their antimicrobial properties, isolates were chosen for the further biochemical characterization [7].

## 4. Results and Discussion

**Table 1.** Presents the biochemical characterization and identification of bacteria producing antibiotics isolated from *Nasutitermes graveolus* and *Microcerotermes tuneri* of Shamawa village.

Isolate	Microscopy	Cit	Ur	Cat	In	MR	VP	Mot
A1	Gram +ve rod (chain)	+	+	+	+	-	+	+
A2	Gram +ve coccus (pairs)	-	+	+	-	-	-	+
A3	Gram -ve rod (single)	-	+	+	-	+	-	-
B1	Gram -ve rod (single)	+	+	+	-	-	-	-
B2	Gram -ve rod (single)	+	+	+	+	-	-	-
B3	Gram +ve coccus (cluster)	+	+	+	-	-	-	+
L1	Gram +ve coccus (chain)	+	+	-	-	-	+	-
L2	Gram +ve rod (pairs)	+	-	+	-	-	+	-
S3	Gram +ve rod (pairs)	+	+	+	-	-	+	+
L2	Gram -ve rod (single)	+	+	+	-	+	-	+
L4	Gram -ve rod (single)	+	+	+	-	-	+	+
S2	Gram +ve coccus (chain)	-	-	+	-	-	+	-
SN2	Gram +ve rod (pairs)	+	+	+	-	-	-	+
SN3	Gram -ve rod (single)	+	+	+	-	-	+	+

**Table 1.** Continued.

Isolate	H <sub>2</sub> S	Gas	Glu	Suc	Lac	Coa	Organisms
A1	-	-	+	+	-	-	<i>Bacillus cereus</i>
A2	-	-	-	-	-	+	<i>Staphylococcus ureae</i>
A3	-	-	+	+	+	-	<i>Escherichia coli</i>
B1	-	-	+	+	+	-	<i>Klebsiella pneumoniae</i>
B2	-	-	-	-	-	-	<i>p. aeruginosa</i>
B3	-	-	+	+	+	+	<i>Staphylococcus aureus</i>

Isolate	H <sub>2</sub> S	Gas	Glu	Suc	Lac	Coa	Organisms
L1	-	-	+	+	-	+	<i>Staphylococcus lentus</i>
L2	-	+	+	-	+	+	<i>Bacillus thuringiensis</i>
S3	-	-	+	+	-	-	<i>Bacillus subtilis</i>
L2	+	-	+	+	+	-	<i>Citrobacter freundii</i>
L4	-	+	+	+	-	-	<i>P. fluorescens</i>
S2	+	-	+	+	+	+	<i>Enterococcus faecalis</i>
SN2	-	-	+	+	+	+	<i>Bacillus megaterium</i>
SN3	-	+	+	+	+	+	<i>Enterococcus cloacae</i>

**Table 2.** Show the frequency and percentage occurrence of antibiotic producing bacteria isolated from *Nasutitermes graveolus* and *Microcerotermes tuneri* nest samples of the study area.

Organisms	Frequency	% Occurrence
<i>Bacillus cereus</i>	6	23.07
<i>Bacillus megaterium</i>	1	3.84
<i>Bacillus subtilis</i>	2	7.69
<i>Bacillus thuringiensis</i>	1	3.84
<i>Citrobacter freundii</i>	1	3.84
<i>Enterococcus cloacae</i>	1	3.84
<i>Enterococcus faecalis</i>	1	3.84
<i>Escherichia coli</i>	1	3.84
<i>Klebsiella pneumoniae</i>	1	3.84
<i>Pseudomonasa aeruginosa</i>	1	3.84
<i>Pseudomonas fluorescens</i>	1	3.84
<i>Staphylococcus aureus</i>	5	19.23
<i>Staphylococcus ureae</i>	2	7.69
<i>Staphylococcus lentus</i>	2	7.69
Total	26	100%

## 5. Conclusion

This study revealed that 26 bacteria isolates with antibiotic producing potentials were identified from 8 mound samples with all yielding growth of which 20 were gram positive and 6 were gram negative therefore. The isolates are *Bacillus spp* *Citrobacter freundii*, *Enterococcus cloacae*, *Enterococcus faecalis*, *E. coli*, *K-pneumoniae*, *Pseudomonas spp*, *S. aureus*, *S. lentus* and *S ureae*. There is an average of 3.25 isolates per sample.

**Table 3.** Showing zone of inhibition of bacterial isolates against *Staphylococcus epidermidis*.

S/NO	Name of Organisms	Zone of inhibition
1	<i>Bacillus cereus</i>	++++
2	<i>Bacillus megaterium</i>	++
3	<i>Bacillus subtilis</i>	+++++
4	<i>Bacillus thuringiensis</i>	+++
5	<i>Citrobacter freundii</i>	++++
6	<i>Enterococcus cloacae</i>	++
7	<i>Enterococcus faecalis</i>	++
8	<i>Escherichia coli</i>	-
9	<i>Klebsiella pneumoniae</i>	++
10	<i>Pseudomonasa aeruginosa</i>	-
11	<i>Pseudomonas fluorescens</i>	-
12	<i>Staphylococcus aureus</i>	++
13	<i>Staphylococcus ureae</i>	++
14	<i>Staphylococcus lentus</i>	+++

The result of this research indicates that termites mound

may be used for medical purposes. Further research needs to be conducted to justify its used medically.

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