

## Original Research Article

<https://doi.org/10.20546/ijcmas.2020.907.251>**Microbial Activity and Cellulose Degraders in Termite Mound Soil****S. Subi and A. Merline Sheela\***

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Soil samples collected from two termite mound soils in Anna University campus, Chennai, Tamil Nadu were characterized for the various parameters such as pH, Electrical Conductivity (EC), Total organic carbon (TOC), Nitrogen (N), Phosphorus(P) and Potassium (K) and microbial activity. The dehydrogenase enzyme activity was found to be 25 and 23mg TriphenylFormosan (TPH) mg/g/h respectively for the two termite mound soils. Further, the presence of cellulose degrading organisms was confirmed by the clearing zones (about 20mm diameter) produced on Agar medium supplemented with Carboxy Methyl Cellulose(CMC) and the population was observed to be 25 and 20 x 10<sup>6</sup> Colony Forming Units(CFU)/ g soil (dry weight basis). By Gram staining, it was confirmed that the bacterial species belonged to Gram negative.

**Introduction**

Termites are the important soil fauna often considered as soil engineers because they are playing an important role in soil forming processes (Jouquet *et al.*, 2016). The occurrence of termite mound is normally found to be in lateritic landscapes of tropical and subtropical regions (Levick *et al.*, 2010). Stability, resistant to erosion (Jouquet *et al.*, 2004), nutrient and mineral richness are the important characteristics of termite mounds (Kebede, 2004). The mounds possess distinct morphology compared with the surrounding soil (Abe *et al.*, 2009). Further, the mounds help to modify the physico chemical characteristics of the soil (Jouquet *et al.*, 2016, Bera *et al.*, 2020).

The termite mound soils are made up of SiO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, Al<sub>2</sub>O<sub>3</sub> (Momah and Okieimen, 2020). It is reported that the mound soil harbored plenty of bacterial species because of the high nutrient content (Enagbonma and Babalola, 2019). Moreover, soil nutrients are accumulated in a termite mound which is important for the ecosystem functioning (Menichetti *et al.*, 2014). Microbial abundance and activity are key indicators for soil quality and measured by different methods (Lori *et al.*, 2017). Dehydrogenases belong to oxidoreductase enzymes group used as an indicator of overall soil microbial activity (Garau *et al.*, 2019). They are occurring within the microbial cells and mediating organic matter degradation (Wolinska *et al.*, 2015) and functioning as an

indicator of soil microbial metabolic activities (Alef and Nannipieri, 1995). Cellulose is one of the important biological compounds found abundantly on the Earth (Beguin and Aubert, 1994). Further, cellulose degrading microorganisms including aerobic and anaerobic bacteria, fungi and actinomycetes are abundant in nature (Jaishree *et al.*, 1985). Termites are playing a key role in degrading the cellulose present in the wood and organic matter in the soil (Nakashima *et al.*, 2002). Hence, the present study was conducted to investigate the bacterial population, the dehydrogenase enzyme activity and the presence of cellulose degrading organisms in termite mound soil found nearby.

## **Materials and Methods**

### **Collection of termite mound soil samples**

Samples were collected from two termite mounds present in Anna University campus, Chennai, Tamil Nadu, India located at 13.04°N and 80.17°E. The soil type of the region is clay with tropical wet and dry climate. The average annual rainfall is 1,400mm. The termite mound from which the samples were collected is shown in Figure 1.

The termite mound soil was collected from middle position of the mound (50 cm and 30 cm from the top for Termite mound 1 and 2 respectively). Two samples (100g) were collected from each mound, air dried, sieved through a 2mm sieve, packed in labeled cover and stored at 4°C for further analysis (Bera *et al.*, 2020).

### **Characterization of termite mound soil**

The pH of the soil was measured in the ratio of 1: 2.5(soil: water). After shaking the mixture in an orbital shaker at 160rpm for 30 minutes, allowed for 30min. pH was measured by pH meter (Elico LI 120)

equipped with combined glass-calomel electrode. The Electrical Conductivity was measured by Digital conductivity meter (Labline) (Jackson 1973). The moisture content of the soil was determined based on (ASTM D2216- 10, 1998). The total organic carbon (TOC) content of the soil was determined by wet oxidation method (Wakley and Black, 1934). The Total Kjeldahl Nitrogen (TKN) was determined following the procedure of Lee *et al.*, (1996).The phosphorus (P) was estimated by extraction of 1.0 g of sample with 50 ml of ammonium fluoride solution. To 5 ml of filtered extract 4 ml of ascorbic acid reagent was added, volume was made up to 10 ml and readings were taken in a Spectronic 20 D + Spectrophotometer at 690 nm and the available P was calculated using K<sub>2</sub>HPO<sub>4</sub> as standard (Olson *et al.*, 1954). Available potassium (K) was estimated with neutral ammonium acetate (200 ml) extraction of termite mound soil in a flame photometer. Values were calculated from KCl standard (Sankaram, 1966).

### **Dehydrogenase enzyme activity**

The procedure of Alef and Nannipieri, 1995 was followed to determine the dehydrogenase enzyme activity. About 6 g of soil was taken in 50 ml serum vials, added with 0.2 g CaCO<sub>3</sub> and thoroughly mixed. The contents of vials were fully saturated to 100 percent Water Holding Capacity (WHC) by adding 1 ml of 3.0 percent aqueous solution of TriphenylTetrazolium Chloride (TTC), 1.0 ml of 1.0 percent sucrose solution and 2.5 ml of distilled water. The vials were sealed and incubated at 37°C for 24 h. The TriphenylFormazon (TPF) formed in each sample was extracted with hot methanol by filtration. The filtration was done until the red color disappeared and the volume was adjusted to 100 ml by adding methanol. The intensity of the red color of the filtrate for

each sample was measured at 485 nm in a Spectrophotometer (Shimadzu UV 1280) using methanol as a blank. The concentrations of Formazon for each sample (i.e., dehydrogenase activity) were determined by referring to a standard curve of the TriphenylFormazon (TPF) in methanol and expressed as mg /g/ h of the sample.

### **Cellulose degrading bacteria**

Initially, the bacterial species were isolated from the termite mound soil by serial dilution (up to  $10^{-7}$ ) and plating method (Singh *et al.*, 2013) in cellulose agar medium composed of  $\text{KH}_2\text{PO}_4$  0.5g,  $\text{MgSO}_4$  0.25g, cellulose 2.0g, Agar 15g, gelatin 0.2g, distilled water 1000ml and pH 6.8 – 7.2) (Gupta *et al.*, 2012). After incubating the plates at 37°C for 48h the colonies were counted and expressed in Colony Forming Units (CFU)/g soil (dry weight basis). The isolated colonies were purified with repeated sub culturing and tested for the presence of cellulose degraders. Subsequently Gram staining (Gram, 1884) was performed to know the type of isolated bacterial species.

Finally, the cellulose degrading ability was confirmed by culturing the isolated bacterial species in Congo red agar medium (Gupta *et al.*, 2012) ( $\text{KH}_2\text{PO}_4$  0.5g,  $\text{MgSO}_4$  0.25g, CMC cellulose (Sigma Aldrich) 2.0g, Agar 15 g, Cong red 0.2g, gelatin 0.2 g, distilled water 1000ml, pH 6.8). After 96h of incubation at 37°C the cellulose decomposition was observed by measuring the diameter of the clearing zone around the colony and expressed in millimeter (mm).

### **Results and Discussion**

The soils collected from the termite mound were characterized and the results are presented in Table 1. The pH of the samples collected from termite mounds 1 and 2 was

found to be 8.1 and 8.2 respectively. The Electrical conductivity (EC) of the termite mound soils 1 and 2 was 0.22 and 0.15 mS/cm respectively. The total organic carbon (TOC) content was 16.80 and 28.40 g/kg for the two samples. The nitrogen (N) (19.60 and 21.20 g/kg), phosphorus (P) (10.2 and 9.76 g/kg) and potassium (K) (26.57 and 10.03 g/kg) contents were analysed for the samples collected from the two termite mounds.

### **Dehydrogenase enzyme activity**

The dehydrogenase enzyme activity of the termite mound soil collected from the Anna University campus was estimated and the results are given in Table 2.

The dehydrogenase enzyme activity of the termite mound soil collected from Anna University campus was found to be 25 and 23 mg TPF/g/h respectively for termite mound 1 and 2.

### **Cellulose degrading bacterial population**

The cellulose degrading bacterial population in termite mound soils was shown in Figure 2

The cellulose degrading bacterial colonies of termite mound 1 was found to be  $25 \times 10^6$  CFU /g soil (dry weight basis) and that of the termite mound 2 was  $20 \times 10^6$  CFU /g soil (dry weight basis).

### **Cellulose degrading ability**

The termite mound soils collected from Anna University campus, Chennai, Tamil Nadu India was analyzed for the presence of cellulose degrading bacterial species. The clearing zones observed in the Carboxy Methyl Cellulose (CMC) Agar medium indicating the presence of cellulose degraders (Figure 3).

**Table.1** Characteristics of Termite mound soil

Parameters	Values *	
	Termite mound soil 1	Termite mound soil 2
pH	8.1	8.2
EC(mS/cm)	0.22	0.15
TOC(g/kg)	16.80	28.40
N(g/kg)	19.60	21.00
P (g/kg)	10.23	9.76
K(g/kg)	26.57	16.03

\*values represent mean of 3 determinations

**Table.2** Dehydrogenase enzyme activity of termite mound soil

S. No	Samples	Dehydrogenase enzyme activity* (mgTPF/g/h)
1.	Termite mound 1	25
2.	Termite mound 2	23

\*values represent mean of three determinations  
(TPF – TriphenylFormazon)

**Fig.1** Termite mounds [(A) Termite mound 1 (B) Termite mound 2] found in Anna University Campus, Chennai (Red arrow indicates the position of sampling)

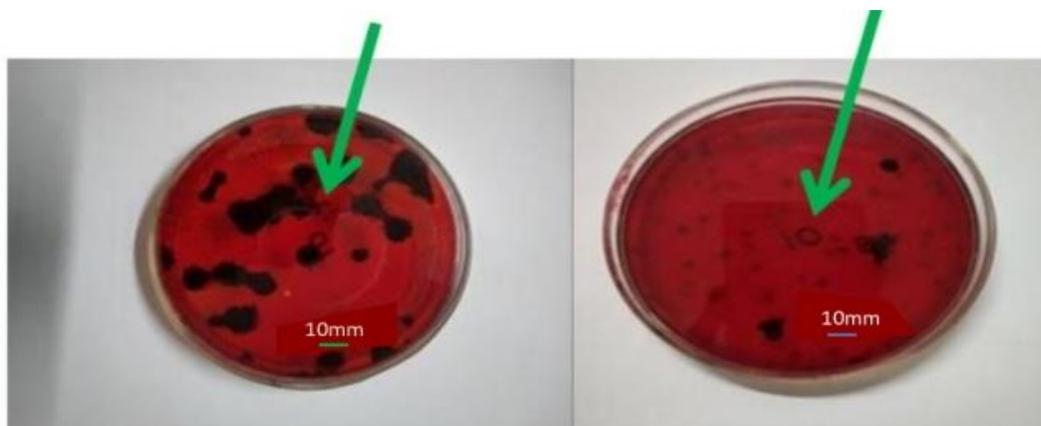


**Fig.2** Cellulose degrading bacterial colonies isolated from termite mound soils (Anna University campus, Chennai, Tamil Nadu, India),



[(A) Termite mound 1(25 x10<sup>6</sup> CFU/g soil) (B) Termite mound 2 (20 x 10<sup>6</sup> CFU /g soil)]  
CFU - colony forming units

**Fig.3** Plates showing clearing zones in Carboxy Methyl Cellulose (CMC) agar indicating the presence of cellulose degraders (Green arrows indicate the clearing zones (10mm) formed by cellulose degraders)



**Fig.4** Photomicrograph of Gram negative cells obtained from termite mound soil (100x)



Further, Gram staining was done to check whether the isolated bacterial species were gram negative or gram positive. On staining, the bacterial cells obtained from the termite mound soil were found to be Gram negative (Figure 4).

In nutrient poor soils termite plays important role in nutrient cycling (Avitable *et al.*, 2016, Lima *et al.*, 2018). This might be the reason for higher N and P contents of the termite mound soils. Further, it has been reported that the P content of the termite mound soil is higher than the surrounding soil, due to the incorporation of feces in the mound material (Lopez-Hernandez *et al.*, 2006). Moreover, the mounds are having more clay content,

which is preventing the loss of P (Oliveira *et al.*, 2012). Soil binding enzymes have been extracted from the termite mound soils (Pariyarath, 2014). Among the oxidoreductases class of enzymes, dehydrogenases is the key enzyme indicating the overall microbial activity of the soil (Salazar *et al.*, 2011). Further, the organic matter decomposition in soil is achieved by these enzymes (Zhang *et al.*, 2019). The dehydrogenase enzyme activity of the fertile soil was found to be > 30 mg TPF/g/h (Kumar *et al.*, 2013), while in the termite mound soil collected from the Anna University campus it was 25 and 23mgTPF/g/h indicating the microbial diversity and microbial activity in termite mound soils. The presence of cellulase

enzyme in the termite mound soil was observed by the formation of clearing zones in agar medium supplemented with CMC. Normally termites are feeding on organic matter rich in cellulose. The bacterial population in termite mound soil is found to be more (up to  $10^6$  CFU/ g sample) (Keya *et al.*, 1982) and the major group is cellulose decomposers (Varma *et al.*, 1994). Furthermore, most recently a plant cell wall degrading enzyme has been reported in gut micro biomes of Neotropical termite species (Victorica *et al.*, 2020).

The bacterial species isolated from the termite mound soils were found to be Gram negative. It has been already demonstrated that in addition to gram positive bacterial species termite gut and mound soil harbored gram negative species (Ramin *et al.*, 2008).

From this study it is concluded that the dehydrogenase enzyme activity was 25 and 23mg TPF/g/h respectively for the two samples analyzed. Further, the bacterial population was 25 and 20CFU/g soil (dry weight basis). The clearing zones with the diameter of 20mm in CMC agar plates indicated the cellulose degrading ability of the bacterial species obtained from the study area. The cellulose degraders were found to be Gram negative bacterial species. However, a thorough study is required to investigate the microbial diversity and microbial enzymes present in termite mound soils available in different regions.

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