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Evaluating lignocellulosic enzyme activity of termite mound soil from different locations of Tamil Nadu

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Abstract
Termite soils provide a very distinct ecological environment which harbors and promotes very specialized cellulolytic and hemicellulolytic microorganisms. This specialized flora enables the termites to feed on hardly degradable polymers such as lignocellulose. The cellulase enzymes synthesized by the microorganisms have a crucial role in recycling C and nutrients and maintaining soil fertility in nature. Hence in the present study, termite mound soils were collected from different locations of Tamil Nadu and their lignocellulosic enzyme activity *viz*., Cellulase, Saccharase and β- glucosidase were assessed to know fertility status of the particular habitats.

Keywords: Termite mound, soil, lignocellulose, enzyme, fertility

Introduction
Cellulose is the most abundant organic compound in the biosphere, synthesized by all higher plants and a wide variety of other organisms, such as fungi, bacteria, invertebrates, and protists (Eriksson *et al*., 1997) [1]. It is the skeletal framework of all higher plants. More commonly, in wood, plant stalks, leaves, cellulose is present with other substances such as lignin and hemicellulose. For instance, wood contains, on a dry basis, from 40 to 55% cellulose, 15 to 35% lignin, and 25 to 40% hemicellulose (Neveil and Zeronian, 1985) [7]. Hemicelluloses are various polymers of hexoses, pentoses, and sometimes, uronic acids. In the pure state, hemicelluloses are easily decomposed. In nature, however, they are frequently complexed with other substances that may make the breakdown more difficult. Lignin is a complex hydrocarbon polymer and its structure is based on the phenyl propanoid unit. This unit consists of an aromatic ring and a three-carbon side chain. It is formed by polycondensation (chemical reaction) of phenolic compounds and free radicals (Paul and Clark, 1989) [10].

Cellulose degradation can be carried out by fungi or bacteria, including mesophilic or thermophilic anaerobic bacteria and mesophilic or thermophilic aerobic bacteria. Enzymatic degradability of cellulose is closely related to its structure, forms, and its association with lignin and hemicelluloses. Lignocellulolytic enzymes constitute a very large group of extracellular proteins mainly, cellulases, hemicellulases, pectinases and ligninases (Mtui, 2012) [6]. Of these, cellulases and xylanases are of significant industrial value and relevance (Favaro *et al*., 2013) [2]. These two groups of enzymes are used significantly in the commercial bio-conversion of lignocellulosic biomass to bio-ethanol (Limayem and Ricke, 2012) [4].

Termites are one of the most important soil insects that efficiently decompose lignocellulose with the aid of their associated microbial symbionts (Ohkuma, 2003) [9]. Termites live in termite mounds, which they build themselves, and these mounds are very predominant in Africa and Asia due to the warmness nature of these two continental regions (Makonde *et al*., 2015) [5]. In nature, termites solely depend on plant litter as their main source of food, however, these insects naturally lack the lignocellulolytic enzymes responsible for the breakdown of lignin and cellulose (Ni and Tokuda, 2013) [8]. Hence, to efficiently utilize lignocellulosic material, termites cooperate with symbiotic fungi and dissimilate a significant proportion of the cellulose (74–99%) and hemicellulose (65-87%) components of lignocellulose they ingest. Consequently termite ecosystem micro flora, greatly contribute to the physical and chemical modification of habitats particularly soils.

Forest soils and cultivable soils contain larger proportion of cellulose owing to the incorporation of leaf litter and other crop residues. These celluloses are eventually degraded by

the action of soil microorganisms, leads to the addition of readily available carbon sources such as glucose and cellodextrins. Termite ecosystem microflora efficiently decomposes the cellulosic material present in the soil and increases the soil fertility. Hence the present study was conducted to explore the lignocellulosic enzymatic potential of termite mound soil, in turn assess the fertility status of soils, collected from various locations of Tamil Nadu.

Materials and Methods

Collection of termite mound soil from different locations of Tamil Nadu

The termite mound soil samples were collected from Thanjavur, Dindigul and Coimbatore districts of Tamil Nadu, using a chisel after removing the adhering dust particles and stored in a pre-sterilized polyethylene bags. The soil samples were transported aseptically to the laboratory for carrying out the further study.

Soil enzyme activity of termite mound soil

Soil enzymes *viz.*, Cellulase, Saccharase and β- glucosidase activity were estimated in the collected termite mound soil and the procedures were provided below.

Cellulase activity

Five grams of sieved (2 mm) soil was placed in an Erlenmeyer flask. 15 ml each of acetate buffer and carboxy methyl cellulose (CMC) solution were added and capped. Eventually, this flask was incubated for 24 h at 50° C and the resulting soil suspension was filtered. The control was prepared by adding 15 ml CMC solution after the incubation but without filtration. 1 ml of the filtrate was diluted to 20 ml with distilled water. 1 ml of the diluted filtrate was pipetted into glass tubes followed by addition of 1ml of reagent A and 1ml of reagent B. The aliquots were mixed well and kept in water bath (100° C) for 15 min. 5 ml of reagent C was added to the solution and mixed well. Finally, the assay mixture was kept aside at 20° C for 60 min for colour development. The optical density was measured at 690 nm against the blank (Schinner and Von Mersi, 1990) [11].

Saccharase activity

Five grams of sieved (2 mm) soil was placed in an Erlenmeyer flask. 15 ml each of acetate buffer and sucrose solution were added, capped and incubated for 3 h at 50° C. After the incubation, the resulting soil suspension was filtered. The control was prepared by adding 15 ml sucrose solution after the incubation but immediately before filtration. 1 ml of the filtrate was diluted to 20 ml with distilled water. 1 ml of the diluted filtrate was pipetted into glass tubes and 1ml of reagent A and 1ml of reagent B were added, mixed well and kept in water bath (100° C) for 15 min. 5 ml of reagent C was added and mixed well. Finally, the assay mixture was allowed to stand at 20° C for 60 min for colour development. The optical density was measured at 690 nm against the blank (Schinner and Von Mersi, 1990) [11].

β- glucosidase activity

0.5 g of sieved (2mm) soil was placed in a 25 ml of volumetric flask and 0.1ml of toluene was added and allowed to stand for 10 min at room temperature. 0.9 ml of distilled water, 1.5 ml McIlvaine buffer and 0.6 ml p-nitrophenyl glucopyranoside were added. The mixture was vortexed and placed in incubator at 30^0C for 1 hr. 8 ml ethanol was added and vortexed for 10 seconds and filtered. 2 ml of 2M Tris buffer was added and swirled for few seconds and the optical density was measured at 400 nm (Koichi Hayano, 1973) [3].

Results and Discussion

Termites are highly beneficial biological agents whose bioturbating and decomposing activities can be managed indirectly with organic matter to enhance primary production. Termite mound soil samples were collected randomly across Thanjavur, dindigul and Western Ghats of Coimbatore districts, Tamil Nadu. Samplings were done in mostly microhabitats of termites and mud galleries and foraging sites in agricultural fields, forest, pastures, along the avenues and also different habitats of termites. Samples were collected from termite mounds situated at various places Thanjavur (5 locations), Dindigul (2 locations) and Coimbatore (3 locations) and their geocoordinates *viz*., altitude, longitude and latitude were recorded using global positioning system (GPS) (table 1). Samples were further analyzed for its enzymatic activity.

Table 1: Collection of termite soil samples from different locations of Tamil Nadu

S. No.	District	Sample	Latitude	Longitude	Altitude
1	Thanjavur	T_1	$10^0$44'33" N	79^0 8'1" E	68m
2		T_2	10^0 44'2" N	79^0 52'9" E	73m
3		T_3	$10^0$43'49" N	79^0 0'39" E	67m
4		T_4	$10^0$44'9.6" N	79^0 6'43" E	79m
5		T_5	$10^0$44'9.5" N	79^0 6'43" E	79m
6	Dindigul	S_1	$10^0$27'29" N	79^0 43'47" E	639m
7		S_2	$10^0$27'1" N	79^0 42'51" E	615m
8	Coimbatore	N_1	$10^0$5'43" N	79^0 43'48" E	611m
9		N_2	$10^0$5'54" N	79^0 44'55" E	600m
10		N_3	$10^0$6'15" N	79^0 44'20" E	575m

Soil enzyme activity of termite mound soil

Soil enzymes key players of soil fertility and indicators of microbial activity. Abundance of enzymes related to lignocellulosic biomass degradation is higher in termite mound soil, due to the action of termite gut microflora and other associative microorganisms in the termite ecosystem. In the present investigation Cellulase activity, Saccharase and β-glucosidase activity of collected samples were analyzed.

Present study reveals that, maximum and minimum enzyme activity was exhibited by the soil samples T1 and N2 which showed cellulase activity of and 231.81 ± 12.35 µg g^{-1} d.wt of soil h^{-1} respectively. This clearly infers that the termite mound soil taken from Thanjavur district has elevated cellulase activity whereas sample from Coimbatore district has the least enzyme activity. We can also confer that the enzyme activities of termite mound soil are bound to the specific region irrespective of the climate zones, termite species involved and altitude (table 2).

Colorimetric estimation of saccharase activity at 690 nm after 3 hours of incubation of termite mound soil implies that maximum enzyme activity was found to be in sample T5 with saccharase activity of 170.15 ± 2.11 µg g^{-1} d.wt of soil h^{-1}. The least enzyme activity was noted in the sample N3 and has the saccharase activity of 112.73 ± 5.57 µg g^{-1} d.wt of soil h^{-1}. From the results we can say that the enzyme activity was higher in the termite mound soils of Thanjavur district and lower enzyme activity was noted in the soils of Coimbatore. Termite mound soils of Dindigul district shows the average saccharase activity. The results are being presented in Table 2. β- glucosidase activity was estimated using p-nitrophenyl glucopyranoside as substrate and the optical density was

measured at 400 nm. Spectrometric observations depict that the sample T5 exhibited maximum β- glucosidase activity of 51.83 ± 0.66 µg g^{-1} d.wt of soil h^{-1} and the least enzyme activity was 31.28 ± 0.71 µg g^{-1} d.wt of soil h^{-1} which was been observed in the sample N2. This results indicate that the termite mound soils of Thanjavur and Dindigul district has the greater β- glucosidase activity above the average and the mound soils of Coimbatore district has the least enzyme activity. The results are being presented in Table 2.

Table 2: Lignocellulosic enzyme activity of termite mound soil from different locations of Tamil Nadu

Sample	Cellulase Activity (µg g^{-1} d.wt of soil h^{-1})	Saccharase Activity (µg g^{-1} d.wt of soil h^{-1})	β- glucosidase Activity (µg g^{-1} d.wt of soil h^{-1})
T_1 - Thanjavur	535.37± 11.3^{a}	134.56 ± 11.86^{bcde}	40.76 ± 0.72^{de}
T_2 - Thanjavur	531.62 ± 29.9^{a}	138.23 ± 4.14^{bcde}	41.82 ± 0.65^{cd}
T_3 - Thanjavur	394.54 ± 19^{b}	152.63 ± 1.31^{ab}	40.53 ± 0.87^{de}
T_4 - Thanjavur	346.13 ± 1.95^{c}	148.48 ± 3.44^{abc}	32.05 ± 0.36^{g}
T_5 - Thanjavur	355.12 ± 8.51^{bc}	170.15 ± 2.11^{a}	51.83 ± 0.66^{a}
S_1 - Dindigul	359.23 ± 13.01^{bc}	146.82 ± 18.17^{abcd}	43.97 ± 1.43^{c}
S_2 - Dindigul	382.91 ± 8.18^{bc}	144.28 ± 2.01^{abcd}	47.33 ± 1.00^{b}
N_1 - Coimbatore	238.56 ± 2.63^{d}	119.87 ± 9.28^{de}	37.96 ± 0.66^{ef}
N_2 - Coimbatore	231.81 ± 12.35^{d}	121.70 ± 5.54^{cde}	31.28 ± 0.71^{g}
N_3 - Coimbatore	265.96 ± 5.6^{d}	112.73 ± 5.57^{e}	35.18 ± 1.43^{f}

Conclusion

Termites play a key role in the carbon cycle of native soil ecosystems. The diet of termites is rich in cellulose and hemicellulose. The micro flora inhabiting termite soil and their gut play a significant role in the dissimilatory activity. They are said to dissimilate a significant proportion of the cellulose (74–99%) and hemicellulose (65–87%) components of lignocellulose they ingest. Hence, termites and their ecosystem microflora have a tremendous ecological impact on the bio recycling of lignocelluloses and present study also confirms this by potential enzymatic activity being present in the analyzed temite mound soil samples.

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