NITROGEN FIXING ABILITY OF SELECTED SUBTERRANEAN TERMITES AND IN THEIR MOUND MATERIALS .

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Abstract: Nitrogen fixation can be considered as one of the most interesting microbial activity as it involves in the recycling of nitrogen on earth and maintains nitrogen homeostasis in the biosphere. Nitrogen input to ecosystem is usually mediated mainly by biological nitrogen fixation by termite gut microbial flora. Most of the subterranean termite species feed on the plant residues with high carbon and nitrogen content. In addition to the carbon mineralization, they have an ecological impact in terms of nitrogen. *T. trinervoides* and *O.obesus* species cum mound materials recorded higher N_2 fixation rate on the intial day and decline in N_2 fixation activity was observed in successive days. More over live termite posses higher N_2 fixation rate than mound materials on the first day of sampling and a decline in N fixation activity was observed in successive days. The ability of termites to contribute the environmental nitrogen have identified termites as ecological keystone species.

Key words: Nitrogen fixation, termites, *T. trinervoides* , *O.obeus*, keystone species.

INTRODUCTION

Soil microinvertebrates are important agents of soil functioning through their bioturbation effects (**Jones *et al.*, 1994**). Termites construct mounds that comprise clay, silt, sand and partially digested plant materials that are cemented together with excreta and saliva usually forming a hard exterior **(Lee and Wood, 1971**). These soil dwelling organisms modify soil properties by displacing soil organic and mineral compounds from one side to another by producing organic aggregates with specific properties (**Jouquet *et al.*, 2000**). Besides soil aggregation and conditioning, subterranean termites *Trinervitermes trinervoides Odontotermes obesus* are among the most important agents of nitrogen economy (**Mettings, 1993)**. The identification of nitrogen fixing bacteria and measurement of their nitrogenase activity has revealed that biological nitrogen fixation accounts for being utilized in agriculture, where eighty percent comes from symbiotic associations and the rest from free living or associative systems like termite microbiota .

MATERIALS AND METHODS

Nitrogen fixation - Acetylene reduction assay

The ability of nitrogenase enzyme within the mound samples to reduce C_2H_2 to C_2H_4 was taken as presumptive evidence for measurement of N_2 fixation rate and laboratory analyses was carried out at Gandhigram Rural University, Gandhigram. . C_2H_4 was measured by using Gas chromatography (Microsam, Siemens) of Pro G analyzer having a Flame ionization detector (FID) equipped with stainless steel column (0.125 inch by 5 feet) containing Porapak N. (80/100 mesh) N_2 was the carrier gas (30 ml/min) and the column temperature was 62°C. C_2H_2 reducing activity in enrichment cultures were measured by injecting 0.3 cm of C_2H_2 into tubes through the rubber septa and then reincubating cultures for 24 h before assaying the presence of C_2H_4 in the gas phase. Assays were performed by injecting mound samples into serum stoppered air filled vials at a volume of 2.5 to 5 % of the vial capacity. C_2H_2 was then introduced in an amount yielding 0.05 nmol of C_2H_4 in the gas phase and

samples were incubated for 1 h at 30°C on a reciprocating shaker operating at 88 oscillations /min. The reaction was terminated with 0.4 ml of 50 % trichloro acetic acid / ml of sample and the head space gas was immediately assayed for C_2H_4 (**Postgate,1972**). For acetylene reduction assay, 2 mg of TMM soil was incubated in each screw cap septum vial for 7 days. Medium used was 10 ml of N free *Azotobacter* medium solidified with 0.3% gellan gum. Then 10 % acetylene gas was injected into 20 ml of gas phase and further incubated for another 1 week.

Assays of live termites were performed in a manner similar to that described previously. Produced ethylene gas was analyzed by gas chromatography, equipped with a glass capillary column Porapak N, under the analytical conditions such as injection at 150°C, detection at 250°C with FID, head gas pressure at 50 kPa and column temperature at 62°C. Representative peak retention time for ethylene is 0.90 min and 1.30 min for acetylene. Using absolute standard curve (peak intensity (y)/n mol for C_2H_4) the total volume of the gas produced in the screw cap septum vial was calculated.

$$\text{Standard amount of ethylene } (E) \text{ in } \mu \text{ mol} = \frac{0.0446 \times Z\ mL}{\text{Peak height in mm} \times \text{attenuation}}$$

$$\text{Amount of ethylene produced in } \mu \text{ mol in the sample} = E \times \text{Peak height of sample ethylene in mm} \times \text{attenuation}$$

$$\text{Activity of nitrogenase} = n \text{ mol or } \mu \text{ mol ethylene per unit sample per unit time}$$

RESULTS AND DISCUSSION

In Dindigul district where mean annual rainfall is low, subterranean termites build mound and harbor in abundance. Harvest termites comprising of *Trinervitermes* sp. and *Odontotermes* sp are the most abundant and conspicuous of termites recorded in sampling blocks of Dindigul district .*T. trinervoides* and *O.obesus* sp are grass harvesting species and soil feeders that feed exclusively on leaf litter and decomposing materials appears to be abundantly present in the sampling blocks. The main food for most termite species is dry plant residues with high carbon and nitrogen content **(Potricus and Breznak, 1981).** The food bolus undergoes a series of digestion by host tissue and bacterial enzymes during its passage through the gut and the rumen diverticulum containing the symbiotic bacterial flora **(Breznak and Brune, 1994**). In addition to the carbon mineralization, they have an ecological impact in terms of nitrogen (**Hong *et al.*, 2006).**

Biological nitrogen fixation refers to the process of microorganisms fixing atmospheric nitrogen, mostly within subsoil involving the conversion of nitrogen to ammonia by microorganisms using a complex enzyme system identified as nitrogenase (**Lindemann and Glover, 2008**). In addition to nitrogen fixation, several methods of nitrogen acquisition and conservation have been identified in termites **(Collins, 1983**), including behavioral mechanisms such as preferential feeding on substrates high in nitrogen and recycling nitrogen among colony members via anal and oral trophallaxis (**Waller, 2000).**

The nitrogen fixation rate in different caste of worker cum soldier and mound materials collected at different depth of two termite different species on 1st, 2nd, 5th and 10th day are given in Table 1. There was notable variation in N_2 fixation rates among the species analyzed ranging from 1.34 mole C_2H_4 g^{-1} h^{-1} in solider caste (TS1) to 2.12 mole C_2H_4 g^{-1} h^{-1} in worker caste (TW2) of *T. trinervoides on* the first day of sampling. In *O. obesus*, the N_2 fixation rate was found to be 1.25 mole C_2H_4 g^{-1} h^{-1} in solider (OS2) to 2.05 mole C_2H_4 $g^{-1}h^{-1}$ in worker caste (OW2) on the first day of sampling.

The amount of N fixed in mound material of *T. trinervoides* at different depth was found to vary from 0.72 to 1.12 mole C_2H_4 $g^{-1}h^{-1}$ on the first day of sampling. Similarly N fixation rate in *O.obesus* mound material at different depth was found to vary from 0.54 to 1.34 mole C_2H_4 $g^{-1}h^{-1}$ on the first day of sampling . Comparatively there was a decline in N fixation rate when analysis was

carried out after 24 h of sampling. (Table.1). Likewise, there was variation in the rate of N fixation between termite mound materials and live termites. The amount of N_2 fixed in *T. trinervoides* was higher than observed in *O. obesus* live species. Nitrogen fixation varied among different caste and rate was significantly higher in the grass feeder than soil feeder. The variation in the nitrogen fixation rate is usually related to functional groups.

Termite Species	Termite caste	N fixation (n mol C_2H_4 g^{-1} h^{-1}) 1st day	2 nd Day	5th day	10 th day	Termite species	Termite caste	N fixation (n mol C_2H_4 g^{-1} h^{-1}) 1st day	2 nd day	5th day	10 th day
Trinervitermes trinervoides	TW1	1.95 ± 0.01	1.86 ± 0.01	1.67 ± 0.01	1.62 ± 0.02	*Odontotermes obesus*	OW1	1.90 ±0.02	1.85 ± 0.01	1.26 ± 0.01	1.22 ± 0.01
	TW2	2.12 ± 0.02	1.98 ± 0.02	1.82 ± 0.02	1.80 ± 0.02		OW2	2.05 ± 0.02	1.98 ± 0.01	1.18 ± 0.01	1.16 ± 0.01
	TS1	1.34 ± 0.01	1.27 ± 0.01	1.33 ± 0.01	1.31 ± 0.02		OS1	1.45 ± 0.02	1.36 ± 0.02	0.92 ± 0.02	0.90 ± 0.02
	TS2	1.36 ± 0.01	1.35 ± 0.01	1.25 ± 0.01	1.16 ± 0.02		OS2	1.25 ± 0.02	1.26 ± 0.02	0.86 ± 0.03	0.84 ± 0.02
TM1	**Mound Soil**	**N fixation (n mol C_2H_4 g^{-1} h^{-1})**				TM 2	**Mound Soil**	**N fixation (n mol C_2H_4 g^{-1} h^{-1})**			
	Outer casing	0.72 ± 0.01	0.65 ± 0.01	0.55 ± 0.01	0.39 ± 0.01		Outer casing	0.54 ± 0.02	0.46 ± 0.01	0.39 ± 0.01	0.23 ± 0.01
	0-10cm	0.87 ± 0.01	0.89 ± 0.01	0.75 ± 0.01	0.51 ± 0.01		0-10cm	0.63 ± 0.01	0.57 ± 0.01	0.47 ± 0.01	0.25 ± 0.02
	10-50cm	1.04 ± 0.01	1.02 ± 0.02	1.01 ± 0.01	0.91 ± 0.01		10-50cm	1.18 ± 0.02	1.17 ± 0.01	0.73 ± 0.01	0.45 ± 0.01
	>50 cm	1.12 ± 0.01	1.08 ± 0.01	1.10 ± 0.01	1.09 ± 0.01		>50 cm	1.34 ± 0.01	1.28 ± 0.01	0.83 ± 0.01	0.32 ± 0.02

Table . 1 Nitrogen fixing ability of selected termite species and termite mounds on different days of incubation

Values are mean ± standard error (n = 3)
TW - *T. trinervoides* worker , TS - *T. trinervoides* soldier
OW - *O. obesus* worker, OS - *O. obesus* soldier

TM1 - *Trinervitermes trinervoides* mound material
TM2 - *Odontotermes obesus* mound material

The identification of nitrogen fixing bacteria and measurement of their nitrogenase activity has revealed that a number of diazotroph species are associated in the gut region**. Peklo (1946)** also reported that termites have endosymbiotic nitrogen fixing bacteria that helps in nitrogen fixation and participates in the important biological process. In the experimental days, a synchronous decrease in nitrogenase activity was observed at 24 hours interval in both species which agrees with the findings of **Brune (2005).** The results of determination of the nitrogenase activity by acetylene reduction assay recorded a higher rate of nitrogen fixation in live termites and lower in termite mound materials. Apparently this can be attributed to a relative stability of the diazotroph community in the termite gut resulting from the reutilization of nitrogen fixers containing faeces accumulated on the permanent substrate.

Conclusion

Biological nitrogen fixation offers a non polluting source of nitrogen and can improve crop production and decrease the global use of synthetic fertilizers. Many termite species utilize dead plant material rich in nitrogen and thus supplement their foods with nitrogen. Since plant materials are carbon rich but nitrogen poor typical symbiotic interactions with termite gut microbes augment

nitrogen economy. These interactions include the recycling of excretory nitrogen and the acquisition of new nitrogen through N_2 fixation. In this regard, nitrogen fixation by termite microbial activity are keys to understand its significance for soil fertility and plant growth.

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