ISOLATION, CHARACTERIZATION AND ENUMERATION OF EFFICIENT MICROORGANISMS IN TERMITE MOUND MATERIALS

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ABSTRACT

This work highlights the isolation, identification and characterization of beneficial microbial population in ten different termite mound materials. As major eco-engineers in tropical ecosystems, termites build biogenic structures with galleries, sheeting's, fungus-comb chambers where the diazotrophs influence nitrogen fixation altering soil mineralogy. Nitrogen fixers strongly influence the physical and chemical properties of soils there by SOM gets altered. In dry land ecosystems, termite mound materials (TMM) are used to enhance soil fertility thus mound soil acts as hotspots for primary production.

INTRODUCTION

Termites are the dominant arthropod decomposers in tropical ecosystems. Termites are major components of detritivore macrofauna feeding on a whole range of living, recently dead plant material in various stages of decomposition (Wood, 1988). Among the soil invertebrates, termites act as ecosystem engineers (Jones *et al.* 1994) which have profound effect on promotion of litter decomposition and the formation of stable pools of soil organic matter.

Termites perform several activities that qualify them as soil engineers. Free living N_2-fixing bacteria are found to be associated in termite gut and mound soil. Biological nitrogen fixation offers a non polluting source of nitrogen and can improve crop production and decrease the global use of synthetic fertilizers. Besides this, termite mound material (TMM) can be used as organic resources to improve soil productivity. It can also counteract land degradation through their soil borrowing and feeding activities.

MATERIALS AND METHODS

Isolation enumeration and characterization of indigenous isolates of *Azotobacter, Beijerinckia, Derxia* sp, Actinomycetes and heterotrophs from different termite mound material

(*Trinervieus trinervoides* and *Odontotermes obesus*) in the vicinity of various blocks of Dindigul district , TamilNadu, India were carried out. The population of the different groups of microorganisms such as bacteria and actinomycetes in various samples were enumerated using standard plate count method (Kannan and Rao, 1996) .

One gram of material from each termite mound soil were suspended in 99 ml of sterile distilled water in a 250 ml Erlenmeyer flask and shaken vigorously for 30 minutes with orbital shaker incubator at 150 rev / min at 24°C to form a uniform solution of 10^{-2} concentration and served as master stock. This stock was subjected to serial dilution with sterile distilled water using a sterile pipette to form 10^{-3} to 10^{-7} concentration. One ml of dilutions 10^{-3} / 10^{-4} were pipetted out to sterile petri plate for the enumeration of bacterial count; the same was repeated with dilutions of 10^{-5}/ 10^{-6} for actinomycetes . The media and growth condition used for the enumeration of Total Colony Forming Units (CFU/ml) of bacteria and actinomycetes are given as follows

Table 1: Media and growth condition

Sl. No.	Microbial group	Isolation media	Dilution factor	Growth temperature (°C)	Period of incubation (in days)
1	*Azotobacter*	Waksman	$10^{-3}/10^{-4}$	37°C	7
2	*Beijerinckia*	Jensens	$10^{-3}/10^{-4}$	37°C	5
3	*Derxia*	Cample's and Doberneir's	$10^{-3}/10^{-4}$	37°C	5
4	*Actinomycetes*	Kenknight's Agar	$10^{-3}/10^{-4}$	37°C	5-7
5	Heterotrophs	Soil extract agar	$10^{-5}/10^{-6}$	37°C	2-3 days

After incubation counts of each microbial groups were noted, average counts and the total population per ml of sample were calculated. The microbial population was expressed as Colony Forming Units (CFU / ml) of the sample. The distinct viable colonies were picked and restreaked on to appropriate agar medium to obtain pure cultures. Each isolate showed its characteristic growth, pigmentation and biochemical reactions. All the bacterial and actinomycetes isolates were identified through morphological and biochemical characteristics. The parameters investigated include colony morphology, gram's reactions, motility, acid production, methyl red reaction, green fluorescent. Voges Proskaur (VP) reaction, catalase reaction, cellulose activity and starch hydrolysis. (Apun., 2000). The test results were compared with that of Bergey's Manual of Determinative Systematic Bacteriology (Holt et.al, 1987) for identification of bacterial and actinomycetes isolates.

RESULTS AND DISCUSSIONS

The isolation, characterization and enumeration of efficient microbes like diazotrophs from termite mound material (TMM) of different species are given in table no. 2 and 3. The total colony forming units of diazotrophs varied across the different samples.

Table 2 : Characterisation of diazotrophs isolated from termite mound material

Colony characters.	*Azotobacter* sp	*Beijerinckia* sp	*Derxia* sp
Cell shape	Ovoid ,rod or cocci appears in pairs	Straight rods with rounded ends	Rods
Colony character	Smooth opaque, convex circular gummy colony with undulate margin	Smooth irregular folded glistening and raised colony with tenacious elastic slime.	White to brown. extremely tenacious growth
Cell size	2 x 1.5µm	2x1 µm	1.0-1.2 µm
Gram reaction	G-ve	G-ve	G-ve
Flagella	Peritrichous	Peritrichous	Peritrichous
Motility	+	+	+
Green fluorescent	+	+	-
Acid production	+	+	+
Cellulose hydrolysis	-	-	-
Starch hydrolysis	+	+	-
Glucose utilization	+	+	-
Sucrose utilization	+	+	-
Maltose utilization	-	-	-
Biotin requirement	-	-	-
Cyst formation	+	+	-
Polysaccharide production	+	+	+

The population of *Azotobacter* sp registered a maximum in TM4, TM5 and TM3 of 8.37 ,8.17 and 8.15 (x10^3 CFU/g) respectively. The population of *Beijerinckia* registered a maximum of 5.67, 5.2 and 5.13 (x10^3 CFU/g)in TM4, TM3 and TM5 material. The *Derxia* load (x10^3 CFU / g) varied from 1.7 to 2.87 in different termite mound analysed. Compared to *Azotobacter* and *Beijerinckia*, *Derxia* load was lesser than other diazotrophs. Highest number of *Azotobacter* and *Beijerinckia* population were recorded in TM5 and TM4. Likewise, the actinomycetes population

registered a maximum load of 12.38 ,12.23 and 12.12 (x10^3 CFU / g) in TM5, TM3 and TM4 respectively. The heterotrophs population were maximum in TM5 and TM3 with 41.67 and 40.27 (x10^6 CFU/g). On analysis, the TM4, TM3 and TM5 were found to possess a maximum diazotroph and microbial load than other mound samples.

Table 3 : Enumeration of microbes isolated from different termite mound at various selected sites

Termite mound	*Azotobacter* sp × 10^3	*Beijerinckia* sp × 10^3	*Derxia* sp× 10^3	Actinomycetes × 10^3	Heterotrophs ×10^6
TM1	5.10 ± 0.20	4.37 ± 0.12	1.23 ± 0.17	9.50 ± 0.01	26.77 ± 0.14
TM2	6.43 ± 0.12	3.60 ± 0.36	1.37 ± 0.12	9.45 ± 0.02	29.77 ± 0.12
TM3	8.15 ± 0.12	5.20 ± 0.08	2.57 ± 0.12	12.23 ± 0.02	40.27 ± 0.12
TM4	8.37 ± 0.12	5.67 ± 0.12	2.87 ± 0.12	12.12 ± 0.07	38.07 ± 0.05
TM 5	8.17 ± 0.12	5.13 ± 0.12	2.50 ± 0.08	12.38 ± 0.04	41.67 ± 0.12
TM 6	4.63 ± 0.17	2.17 ± 0.12	1.35 ± 0.01	10.17 ± 0.15	34.17 ± 0.24
TM 7	6.57 ± 0.12	4.43 ± 0.12	1.17 ± 0.12	8.86 ± 0.01	27.57 ± 0.34
TM 8	5.47 ± 0.12	ND	1.47 ± 0.12	8.23 ± 0.01	26.17 ± 0.12
TM 9	5.47 ± 0.12	1.80 ± 0.08	ND	8.77 ± 0.12	24.30 ± 0.08
TM 10	4.23 ± 0.12	2.67 ± 0.12	1.77 ± 0.12	8.10 ± 0.01	25.60 ± 0.36

ND - Not detected Values are mean ± standard error (n = 3)

Microorganisms are the dominant biotic structural components in soil and have higher biomass specific activities. The list of N2 fixing bacteria associated with non legumes includes species of *chromobacter*, *Acetobacter*, *Azotobacters*, *Beijerinckia*, *Bacillus*, *Enterobacter*, *Erwinia*, *Derxia* and *Rhodospirillum* (Wani,1990). Although many genera and species of N_2 fixing bacteria are isolated from the rhizosphere of various cereals, mainly members of *Azotobacter* and *Azospirillum* genera have been widely used to increase the yield of cereals and legumes in green house and under field conditions.

Diazotrophs are found in a wide variety of habitats including free living forms in soils, water, termite mound material (TMM), cyanobacterial symbioses with various plants and root nodule symbiosis with legumes (Dixon and Kahn, 2004). *Azotobacter* is a free living nitrogen fixing bacterium, which is used as a biofertilizer in the cultivation of most crops. It has several metabolic capabilities with highest metabolic rate to fix 20-60 kg nitrogen per hectare of land annually. *Azotobacter* is the most common biofertilizer for plants like maize, wheat, sorghum and rice which produces some plant growth promoting metabolites, nutrients to plants, enzymes, different growth hormones like IAA, auxins, gibberllins, cytokinins , vitamins and siderophores. It is probable that non symbiotic nitrogen fixers of the microbial population are widespread and abundant in termite mound material (TMM) which accounts for higher nitrogen fixation and crop yield.

CONCLUSION

Non symbiotic diazotrophs in termite mound can promote economic and environmental benefits including increased income from high yield, reduce fertilizer cost and reduce leaching of NO_3 to ground water. In this regard, diazotrophic diversity of termite mound material (TMM) and characterization are keys to understand the significance of nitrogen fixers as biofertiliser. Various environmental determinants and factors inherent to termite biology influence microbial distribution and play a role in ecosystem processing influencing soil fertility by providing beneficial role in agriculture.

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