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Biological Exopolysaccharide Producers in Subterranean Termite Gut

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ABSTRACT

Subterranean termites *Odontotermes* sp. and *Trinervieus sp.* modify the soil properties through their mound- building activities, subterranean galleries, storage chambers, aggregate formation, aeration, organic content and soil fertility. Gut of subterranean termites are structured habitats with numerous microniches created by a combination of host and microbial activities with rich population of diazotrophs. The *Azotobacter* isolates of *Odontotermes* sp. and *Trinervieus sp.* worker caste showed a positive response in the production of enzyme dehydrogenase and biological exopolysaccharide. Biological exopolysaccharide producers influence soil quality cum plant growth increasing agricultural productivity.

Introduction

Soil microinvertebrates are important agents of soil functioning through their bioturbation effects. Subterranean termites *Odontotermes* sp. and *Trinervieus sp.* 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). Termites secrete a large proportion of extracellular polysaccharides (EPS) which can effect soil aggregation, moisture and stability.

Materials and methods

A field survey was conducted at various sampling blocks of Dindigul district. Subterranean Termite mound of *Odontotermes* sp. and *Trinervieus sp.* in different transect was documented. Termites were identified to genus level through the dichotomic keys of Fontes and Constantino (1983). The different caste of termite was identified and sorted out. The free living beneficial microbes *Azotobacter,* present in the gut region of worker, soldier and queen termites of *Odontotermes* sp. and *Trinervieus sp.* were isolated. The individual colonies developed in the plates were transferred for subculture. Diazotroph isolates are subjected for screening and production of exopolysaccharide and dehydrogenase enzyme.

Extraction of Polysaccharide

Polysaccharide was extracted by the modified method of Evans and Linker (1973). The bacterial culture on agar plate was scraped aseptically and transferred into 25 ml of sterilized saline and stirred to get a uniform mixture. This suspension was centrifuged at 1000 rpm for 30 minutes. The precipitate was discarded and supernatant was centrifuged again for half an hour. The precipitate was again discarded. To the supernatant three volumes of 95% ethanol was added with stirring to precipitate the polysaccharide. The precipitate was washed with absolute alcohol and then dried to remove ethanol vapors. Dried precipitate was further purified in 0.1M tris buffer pH 7.4 at 5 mg per ml and optical density was measured at 630 nm using spectrophotometer (Spectronic, model SI-104) after incubation at 37°C for 48 hours.

Production of Dehydrogenase

Quantative measurement of dehydrogenase secreted by bacterial isolates was determined by the procedure described by Casida *et al.* (1964). Diazotroph isolates and 0.2 g of $CaCO_3$ were thoroughly mixed and dispensed in 10 test tubes. Each tube was added with one ml of 1.5 percent aqueous solutions of 2, 3, 5 tripheny ltetrazolium chloride (TTC), one ml of one percent glucose solution and eight ml of distilled water which was sufficient to leave a thin film of water over the soil layer. The test tubes were stoppered with rubber bands and incubated at 30°C for 24 hours. At the end of incubation, the contents of the tube were rinsed down in to a small beaker and converted into slurry by adding 10ml methanol, the slurry was filtered through whatman filter paper no.42. Repeated rinsing with methanol was continued till filtrate ran free of red colour. The intensity of red colour was measured at 485 nm against a methanol blank using spectrophotometer (Spectronic, model SI-104).The results were expressed in microgram of triphenyl formazon (TPF) formed per ml of culture per day.

Results

Different *Azotobacter* strains isolated from termite gut of two species of worker, soldier and queen are capable of synthesizing exopolysaccharides and dehydrogenase. The amount of polysaccharide produced by different *Azotobacter* strains isolated from *Odontotermes* sp. and *Trinervieus sp* varied from 0.30-1.22 mg/ml. Highest amount of polysaccharide was recorded by *Azotobacter* strains (AZT1) isolated from *Trinervieus sp* worker (TW1) and lowest amount was registered by *Azotobacter* isolate (AZT9) of *O. obesus* soldier (OS2). The amount of dehydrogenase produced by different *Azotobacter* strains isolated from

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Odontotermes sp. and *Trinervieus sp* varied from 5.41-9.53 µg TPF/ml (Table 1). On comparing two species, the *Azotobacter* strain (AZT1) of *Trinervieus sp* showed higher efficiency in dehydrogenase and polysaccharide production. but higher amount was registered in the gut of worker compared to other caste. Therefore polysaccharide and dehydrogenase production was higher in worker caste than soldier and queen of the two species studied. From this, it is evident that the *Azotobacter* strains of *Trinervieus sp* worker (TW1) were efficient in synthesis of dehydrogenase and polysaccharide secretion than other caste.

Table 1. Production of dehydrogenase and polysaccharide by different *Azotobacter* strains isolated from two termite species of different caste.

Termite	caste	*Azotobacter* isolates	Dehydrogenase(µg TPF/ml)	Polysaccharide(mg/ml)
Trinervitermes sp	TW1	AZT1	9.53 ± 0.01	1.22 ± 0.02
	TW2	AZT2	8.87 ± 0.01	0.82 ± 0.01
	TS1	AZT3	8.87 ± 0.01	0.82 ± 0.01
	TS2	AZT4	7.62 ± 0.01	0.91 ± 0.01
	TQ	AZT5	5.90 ± 0.02	0.57 ± 0.01
Odontotermes sp	OW1	AZT6	8.84 ± 0.01	0.86 ± 0.01
	OW2	AZT7	9.47 ± 0.01	1.17 ± 0.01
	OS1	AZT8	6.88 ± 0.01	0.41 ± 0.01
	OS2	AZT9	5.41 ± 0.01	0.30 ± 0.02
	OQ	AZT10	5.51 ± 0.02	0.51 ± 0.02

Values are mean ± standard error (n = 3)
TW-*Trinervieus* worker
TS-*Trinervieus* soldier
TQ - *Trinervieus* queen
OW - *Odontotermes* worker
OS - *Odontotermes* soldier.
OQ - *Odontotermes* queen
AZT - *Azotobacter* strains

Discussion

Termites are beneficial biological agents whose bioturbating and decomposing activities can be managed indirectly with organic matter to enhance primary production (Jones *et al.,* 1994). Biodiversity of Subterranean termite in Dindigul district revealed that there are more number of mounds colonized by *Odontotermes* and *Trinervieus* species. Free living N_2 fixing bacteria belonging to the genera *Azotobacter, Beijerinckia,* and *Azospirillum* are found to be associated in termite gut and termite mound materials.

Subterranean Termites are beneficial biological agents whose gut portion are harboured by a number of diazotroph species which indirectly influence the primary production. These diazotrophs isolates are capable of extracellular polysaccharide productions. The approximate composition of the organic matter was 40% carbohydrate all of which occur as polysaccharides which on hydrolysis were shown to be dominated by glucose. The soil dehydrogenase activity increased with the increase in organic carbon and higher microbial population. Some activities of pyruvate dehydrogenase were detected in gut-free extracts of the lower termite (Nihorimbere *et al.,*2011). It was assumed that the depolymerization products of polysaccharides are absorbed by the midgut epithelium and reach the hindgut. In view of the dehydrogenase activity in termite gut tissue, polysaccharides was oxidized into pyruvate by the enzymatic activity and subsequently converted to acetate by the gut microbiota.

Extracellular polysaccharides are those carbohydrate polymers, which appear outside and normally unbind to the cell walls of microorganisms. Bacterial polysaccharides are normally secreted in the form of capsules, slime or extra cellular gum having insoluble hydrophilic gel property (Lawson and Sutherland, 1978). *Azotobacter* sp. has the property of EPS production in high temperature and desiccation conditions. Extracellular polysaccharides (EPS) are a large portion of the insoluble soil components and can modify the physical properties of the soil such as aggregation and stability. The accumulation of EPS in soil can increase the soil aggregation and subsequently reduce the oxygen diffusion through densely packed soil material (Miller and Donahue, 1995).

Conclusion

The dominant species *Trinervieus* and *Odontotermes* worker community guts with defined microhabitats, and the high specific activities of polysaccharide production by the microbial populations make termite guts for studying functional interactions with soil conditioning and plant productivity. Various environmental determinants and factors inherent to termite biology influence termite distribution and play a role in ecosystem processing influencing soil fertility by providing beneficial microbes for agriculture.

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