Available online at www.elixirpublishers.com (Elixir International Journal)

Agriculture

Elixir Agriculture 39 (2011) 4774-4776

ISSN: 2229-712X

Potentials of fungus cultivating termites in a tropical ecosystem

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ARTICLE INFO

Article history:

Received: 14 July 2011;

Received in revised form:

21 September 2011;

Accepted: 28 September 2011;

Keywords

Macrotermes,

Termite symbiosis,

Fungal comb,

Extracorporeal digestive system.

ABSTRACT

Termites are significant agents behind organic decomposition in or at the surface of the soil. They thrive in great abundance in terrestrial ecosystems and play important role in biorecycling of lignocellulose... Macrotermitinae are the dominant termite family which cultivate fungal combs within the mound chamber. The termites and fungi, form a agricultural beneficiary relationship which is one of the most spectacular example for mutualistic symbiosis The key activities attributed to the fungal partner in this mutualistic symbiosis are extensive delignification of the substrate and the conversion of plant fiber to fungal biomass .The fungal population aids in the decomposition of cellulose and supply other nutritional requirements for the termite.

Introduction

In tropical and many subtropical regions termites are the most important soil fauna both in terms of numbers and their effects on the soil. Fungus-growing termites (Isoptera, Macrotermitinae) play an important role in tropical ecosystems in modifying soil physical properties. Most of the literature regarding the impact of termites on soil properties refers to termite epigeous mounds. Their nests are made of several hundreds of units, so-called “fungus-comb chambers”, where the exosymbiotic association occurs. Together with their microbial symbionts and fungal comb they efficiently decompose lignocellulose. There is some nitrogen fixation that also takes place.. Due to their digestive ability and their huge abundance, termites have a tremendous ecological impact on the biorecycling of organic matter .

Materials and methods

Isolation and screening of fungus in termite nest material. Termite mound were excavated and nest material were collected from four different termite nests at different locations in dindigul district , south India. The fungal species present in the different samples were isolated and screened using standard plate count method (Kannan, 1996; Nagarathnam et al., 2000; Hart, 2002

One gram of material of each sample was suspended in 99 ml of sterile distilled water in a 250 ml Erlenmeyer flask and shaken vigorously for 30 min with orbital shakes incubator (Remi instrument Ltd., model: CIS – 24) at 150 rev/min at 4°C to form a uniform solution of 10^{-2} concentration and served as master stock. This stock was subjected to decimal dilution using sterile pipettes to form 10^{-2} to 10^{-3}concentration.

One ml of dilutions $10^{-3}/10^{-2}$ was pipetted to sterile petriplate for the isolation of fungi. The media and growth condition used for isolation of fungi are as follows:

The well isolated and the predominant zone forming fungi were picked out from the mother plate and spotted on the Rose Bengal agar Plates. Then the plates were incubated at room temperature and well grown organisms were maintained in the slants as pure culture,. All the fungal isolates were identified based on their colony characteristics and the microscopic characteristics such as hyphal & reproductive structure after staining with Lactophenol cotton blue (Comsch et al., 1980); Aneja, 2001; Alexopoulos & Mims, 1983).

Ten gram of fresh fungal comb material was grounded to a powder, dissolved in 100 ml distilled water and centrifuged. The supernatant was taken for analyzing the pH, and enzyme activity such as xylanase, and cellulose .. The moisture content was also recorded by measuring the weight of the comb after complete drying and getting a constant weight in 105^0C oven. Total nitrogen amount of the comb was measured following the Modified Kjeldhal method. Soluble protein content was measured following the method of Lowry *et al.* (1951) with BSA (bovine serum albumin) used as standard. The concentration of reducing sugar in the comb was measured by a colorimetric assay using xylose as standard. .

Xylanase activity was determined by measuring the amount of reducing sugar released from xylan following the dinitrosalicylic acid (DNS) method (Miller, 1959). The reaction contained 1% birch wood xylan in 0.2 M acetate buffer pH 5.0 and 100 µl enzyme preparation. After 10 minute incubation at 50^0C, the reaction was stopped by adding 2 ml DNS reagent followed by boiling for 5 minutes. Absorbance was measured on spectrophotometer at 540 nm against a reagent blank. One unit (U) is defined as the amount of enzyme that releases1µ mol of reducing sugar equivalent to xylose perminute. The amount of xylanase was expressed as U/g.. cellulase activity was estimated using 0.5% CMC dissolved in acetate buffer as substrate. One unit (U) of cellulase activity was defined as the amount of enzyme that releases 1µ mol reducing sugar equivalent to glucose per minute.

Sl. No.	Microbial group	Isolation media	Dilution factor	Growth temperature ((°C)	Period of incubation (in days)
1	Fungi	Martin’s Rose Bengal Agar	$10^{-3}/10^{-2}$	28°C	3-5

Discussion

Microorganisms are the dominant biotic structural components in soil and have high biomass specific

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Activities. Termites feed on the food which contains lignified cellulose (Sands, 1977). Cellulose is consumed and digested with then help of cellulolytic gut microflora of the termite (Breznak, 1982). However, cellulases secreted by those gut microbes are not capable of digesting the native cellulose because cellulose microfibriles are embedded in a sheath of lignin and hemicelluloses making the cellulose inaccessible for cellulases (Amare Gessesse, 1998). However, no lignin degraders and very few xylanolytic microorganisms have been reported in the termite gut (Ajit *et al*., 1994). This shows that the termite depends mainly on fungal population for delignifying cellulose.

However, to date only few fungi are known to be associated with termites. The various fungal species (9) found in the termites sample material were identified on the basis of colony morphology ,microscopic characteristics and were listed in the table 2. Among them, strongly cellulolytic fungi were represented by species of the genera *Aspergillus, , Curvularia,* and *Trichoderma*. The genera aspergillus growth dominated the other fungal population.

This diverse group of fungi utilizes cellulose for its carbon and energy sources (Turner ,2002). It has also been proposed that fungi are the main agents of cellulose degradation in humid soil while bacteria are of greater significance in semi arid localities (Alexander, 1976). The primary role of cellulolytic microorganisms in both the natural environment and agriculture is in the decomposition of organic matter, a process that can be optimized during the composting of plant residues (Hart *et al.*, 2002).

Properties of the termite comb

The properties of termite comb showed an acidic pH and moisture range between 48.5 to 54.6 % .The amount of carbon in the comb material was found to be around 2.35 % and 2.73% . The load of nitrogen does not exceed 1.68 %. The calculated C/N ratio showed a minimum of 1.45 % and maximum of 1.64 % . The organic matter was estimated by multiplying the carbon content in to 1.72. The amount of reducing sugar level confirmed the fungi as an extracorporeal digestive system that converts undigested woody material in plants into higher quality oligosaccharides and more easily digestible complex sugars. The extensive decomposition of lignocellulose by fungus-growing termites was due to their enzyme composition such as xylanes and cellulases . However the amount of cellulases estimated was in significant but ,the amount of xylanses showed their dominance in degrading lignin of polysaccharide components These ecosystem engineers consume more than 90% of dry wood in some arid tropical areas and directly mineralize up to 20% of the net primary production in wetter savannas (Abe et al. 2000). Thus these organisms play a predominant role in the mineralization of organic compounds.

Conclusions

The results presented confirm the overall view about the fungal role as symbiotic organisms in degrading organic matter within the termite mounds.. However, the estimates provide some useful insight into the relative importance of the biological activity of subterranean and epigeous termites on tropical regions .However a detailed study should be put forth to analyze the importance of macrotermes as efficient degraders .

References

Abe T, Bignell DE, Higashi M (2000) Termites: evolution, society, symbiosis, ecology. Kluwer, Netherlands

Ajit,V. Ballakrishan, K., Jaishree, P., Shailendra, S. and Helmut, k. (1994).

Lignocellulose degradation by microorganisms from termite hills and termite guts:A survey on the present state of art. *FEMS Microbiol*. *Rev*. 15: 9 – 2

Amare Gessesse (1998). Purification and properties of two thermostable alkaline xylanse from alkaliphilic *Bacillus species*. *Appl. Environ. Microbiol*. 64: 3533-3535.

Breznark, J. A. (1982). Intestinal microbiota of termites and other xylophagous insects. *Annu . Rev. Microbiol*. 36: 323 – 343.

Hart T.D., F.A.A.A.M. De Leij., G.Kinsey and J.M.Lynch (2002). Strategies for isolation of celluloytic fungi for composting of wheat straw world journal of Microbiology & Biochemistry. 18: 471-480.

Lowry, O. H., Rosebrough, N. J., Farr, A. C. and Randall, R .J. (1951). Protein measurement with folin phenol reagent. *J. Biochem.* 193:265–2

Miller, L., (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31: 426 -428.

Sands, W.A. (1977). The role of termites in tropical agriculture. *Out look Agri*. 9: 136 – 143.

Turner, J. S. (2002). A super organism’s fuzzy boundary. *Nat. Hist*. 11:62-67.

Table : 2 Isolated and Screened fungal species in the macrotermes -termite nest material

Sl.no	Colony morphology	Microscopic characteristics	Fungal species
1	Cottony colony with black spores	Conidia with spherical spores	Aspergillus niger spp.1
2	Cottony colony with black spores	Conidia with spherical spores	Aspergillus niger spp.2
3	Greenish and velvety colony	Conidia in globose	Aspergillus niduam
4	Greenish colony with radiated ring	Conidias in long chain on repeatedly branched conidiophores resembling a brush like head	Pencillum oxalicum
5	Grey greenish colony with restricted growth	Conidia in globose	Penicillum corylophilum
6	colony spreading with tuff base	Conidia ellipsoidal and smooth	Penicillum funiculosum
7	Light green colony, center raised smooth	Conidia in balls	Trichodermaspp 1
8	Light green colony, center raised smooth	Conidia in balls	Trichoderm spp 2
9	Colony grey in color with irregular borders and cottony growth	Conidia curved and cells with middle septum	Curvularia pallescens

Table 3 Physico -chemical properties of the termite - fungal combs studied in four sites

Properties	Fnm1	Fnm2	Fnm3	Fnm4
pH	4.8	4.7	4.9	4.7
Moisture content	54.2 %	54.6 %	50.2 %	48.5 %
Total carbon	2.35 %	2.56 %	2.45 %	2.73 %
Total nitrogen	1.47 %	1.56 %	1.68 %	1.62 %
C/N ratio	1.59	1.64	1.45	1.68
Organic matter	4.04	4.40	4.21	4.69
Reducing sugar	385 µg / g	362 µg / g	376 µg / g	355 µg / g
Total soluble protein	30.5 5mg/g	28.2 5mg/g	29.5 5mg/g	28.5 5mg/g
Xylanase	8.15 U/g	8.35 U/g	8.42 U/g	8.75 U/g
Cellulase	0.76 U/g	0.64 U/g	0.56 U/g	0.60 U/g