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Antibiotic Properties and Characterization of Termitarium of Common South Indian Termites

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ABSTRACT

Termitarium are elaborate structures made using a combination of soil, mud, chewed wood, cellulose, saliva and faeces by the eusocial insect, termites. It is highly rich in organic matter. Some termite sp. maintaining fungal growth that is fed on plant dead matter, providing a nutritive mycelium on which the colony then feeds. Termite mounds are consist of maze of tunnel that provide air conditioning and regulating CO_2 and O_2 balance, further facilitating termites to travel through the nest. In the traditional medicinal periods, termite mound soil used for curing skin related diseases. Present experiment was carried out to find the antibiotic property of the termitarium (soil). The soil sample was extracted with three different solvents like water, ethyl acetate and petroleum ether and tested against following pathogens such as *Pseudomonas putida*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Escherichia coli*, *Bacillus* sp. and *Citrobacter* sp as well as tested against the culture isolated from blister and mold infection. Water extract of termitarium showed significant antibacterial activity against *P. putida*, *L. acidophilus*, *Bacillus* sp. and isolate from blister infection. Petroleum ether extracted sample was shown significant antibiotic action against *S. mutans* and *L. acidophilus*. Similarly ethyl acetate extract performed an effective action against *E. coli* and isolate from mold infection.

Keywords: Termitarium, extraction, antibacterial action, pathogen

INTRODUCTION

Termites are terrestrial, social insects which inhabit around two-thirds of land source between 45^{o} North and South latitude (Wood and Thamos, 1989), and also present in temperate zones (Marini and Mantovani, 2002). Highly evolved termite species have difference from lower evolved termites by means of poor or absence of symbiotic association with flagellates in their hindgut. The absence of such microbial association leads to limit food variation and evolutionary feeding habit changes (Noirot, 1992).

Termites in some regions notably in tropical savannas are constructing extremely huge mounds (termitaria) for their colonies dwelling places. Termites belongs to *Microtermitinae* are good termitarium builders in tropical Africa have raise 30 diameters mound (Kone et al, 2013). They feed on cellulose, directly from plants, dead or alive, or indirectly from fungus arising from decaying plant material within mounds (Resh and Carde, 2009). The presence of growth promoting rhizobacteria in the mount soil is enriching solubilization of potassium, mineralization of phosphate and plant pathogen suppressing antibiotics (Enagbonma and Babalola, 2019).

Macrotermes bellicosus (termites) is another type of termites present in Africa and Asia make a hole in the soil called copularium facilitating symbiotic association with fungus contribution to impart metabolites (Ntukuyoh et al. 2012). In termite saliva there are cellulose digesting enzymes: a β-1-4-glucanase that brings about the initial splitting of the polymer, and β- glucosidase that degrades the resulting cellobiose to glucose (Nakashima *et al*., 2002; Tokuda *et al*, 2005). The other geochemical present in termitarium soil was Au, Ag, Cu, Zn, Co, Mn, Fe and Ni (Mugerwa, 2015). Traditional peoples are using the termitarium soil sample for curing skin diseases, therefore present study aimed to find antibiotic property of the termitarium soil sample.

MATERIALS AND METHODS

3.1. Sample collection and analyses microorganism present

The termitarium sample was collected from our college premises. A series of dilution were made up to 10 fold to reduce the microbial populations in the sample. Spread plate technique was used to isolate the organisms by spreader 500 µl of diluted samples in the Nutrient Agar and Potato Dextrose Agar. The inoculated plates were incubated at 37˚C for overnight. Single developed colony was picked on the nutrient agar and sub-cultured in Nutrient Broth for further process. The shape, color and motility of the colonies are examined under the phase contrast microscope stained with Gram stain and Endospore Stain. Isolates were biochemically analysed for Enzyme activity (Starch hydrolysis, Casein hydrolysis), Indole Methyl Voges-Proskaur Citrate (IMViC) test, and Hydrogen sulphide test. All these tests were used for identification microbial isolates from termitorium soil according to Bergey's manual of systemic bacteriology (Claus and Berkeley, 1986).

3.2. Antibacterial assay

The antibacterial was tested against the following strains *Escherichia coli* (MTCC 40), *Pseudomonas putida* (MTCC 7173) *Streptococcus mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307) and the isolate from soil *Bacilli species*. For antibacterial assay the organisms grown in respective medium as given below

STRAIN	CULTURE MEDIUM
Escherichia coli	Nutrient broth
Pseudomonas putida	Nutrient broth
Streptococcus mutans	LB broth
Lactobacillus acidophilus	MRS broth
Bacilli species	Nutrient broth

The antibacterial activity was carried out against the organisms isolated from the mold (Fig. 1) and blister (Fig. 2) infection. The well diffusion method is used for the antimicrobial examination. Wells loading samples were prepared by mixing 5g of termitarium sample in 10 ml of distilled water, petroleum ether and ethyl acetate respectively. 20µl of each prepared solution were added in well respectively and incubated at 37˚C.

Fig 1: Mold infection zone

Fig 2: Blister infection zone

Test 1- 5 g of termitarium + 10 ml of distilled water.

Test 2- 5 g of termitarium +10 ml of petroleum ether.

Test 3- 5g of termitarium +10 ml of ethyl acetate

After 24hrs of incubation the zone of inhibition were measured and also compared with commercially available antibiotic, Oxytetracycline hydrochloride which is used as positive control.

RESULTS AND DISSCUSION

Microbes in termitarium

After 24 hrs of incubation the nature of colonies formation in nutrient agar and Potato Dextrose Agar (PDA) medium were observed. The external morphology of the colonies appeared in nutrient agar is like small dots, clever shape with different dimensions (Fig.3). In PDA medium growth of fast grown fungal species is observed (Fig. 4). These findings are reporting that termites have good symbiotic association with anaerobic bacteria in their gut and association with nonpathogenic fungal species as the substrate for the termites. These ecofriendly associations have a significant impact on plant growth and cause number beneficial action on human and environmental health (Ayitso et al. 2015).

Fig 3: Colony formation from inoculation of inoculate prepared from termitarium sample

Fig 4: Growth of fungal species isolated from termitarium sample

Table 1: Characterization of termitarium isolate

Morphological/Physiological/ Biochemical Characteristics	Result	Description
Cell shape	Rod	Long and rod shaped cells
Gram stain	Negative	Gram negative not contain thick cell wall
Spore formation	Non spore	Not have the capsule coat and non-spore cells
Motility	Motile	Motile by the mode of flagella on the cell
Starch hydrolysis	Negative	Not to degrade the starch reveals the absence of amylase
Casein hydrolysis	Negative	Absence of the proteases
Bubble formation	Positive	Ferment the carbohydrates and produce gas as the byproduct
Indole production test	Positive	Able to convert tryptophan into indole

Methyl red test	Positive	Indicate the production of acid
Voges- Proskauer test	Negative	Not able to form acetylmethylcarbinol
Citrate utilisation test	Positive	Utilise citrate as a carbon source
Hydrogen sulphide test	Positive	Contain sulfates to serve as the substrate for detecting sulfide production

The biochemical characterization (Table 1) revealed that the organism isolated from termitarium sample belongs to Proteobacteria and Actinobacteria species and its exact characteristic features to be analyzed in future work. These organisms effectively using organic matter present in the soil and convert usable metabolized products released in termite mound soil (Subi and Merline Sheela, 2020).

Antibiotic assay

The antibacterial activity of extract prepared from termitarium soil against the selected organism showed significant action (Table 2). Water extract showed significant antibacterial activity against *P. putida* (Fig. 5a), *L. acidophilus* (Fig. 5e), *Bacillus* sp. (Fig. 5f) and isolate from blister infection (Fig. 5g). Petroleum ether extract performed good significant action against *S. mutans* (Fig. 5b) and *L. acidophilus*. Similarly ethyl acetate extract performed notable effective action against *E. coli* (Fig. 5c), isolated bacterial species from soil (Fig. 5d) and isolate from mold infection (Fig. 5h). The significant inhibition activity against the selected organism is due the presence of nematicidal, antiviral and antimicrobial substances in the termitarium (Chauhan et al. 2017).

Table 2: Antibacterial activity of water, petroleum ether and ethyl acetate extract of termitarium sample.

Organism	Extract sample	Growth zone Diameter (cm)
Pseudomonas putida	Control	1.57
	Water	0.22
	Petroleum ether	0.55
	Ethyl acetate	0.53
Streptococcus mutans	Control	2.34
	Water	0.61
	Petroleum ether	0.05
	Ethyl acetate	0.93
Escherichia coli	Control	2.52
	Water	0.51
	Petroleum ether	1.02
	Ethyl acetate	0.51
Soil isolated	Control	1.64
	Water	0.54
	Petroleum ether	0.43
	Ethyl acetate	0.66
Lactobacillus acidophilus	Control	1.10
	Water	0.59
	Petroleum ether	0.51
	Ethyl acetate	0.87
Bacilli species	Control	3.06
	Water	0.73
	Petroleum ether	1.52
	Ethyl acetate	1.25
Blister isolate	Control	2.32
	Water	1.06
	Petroleum ether	2.12
	Ethyl acetate	1.63
Mold infection isolate	Control	2.36
	Water	1.54
	Petroleum ether	1.56
	Ethyl acetate	0.90

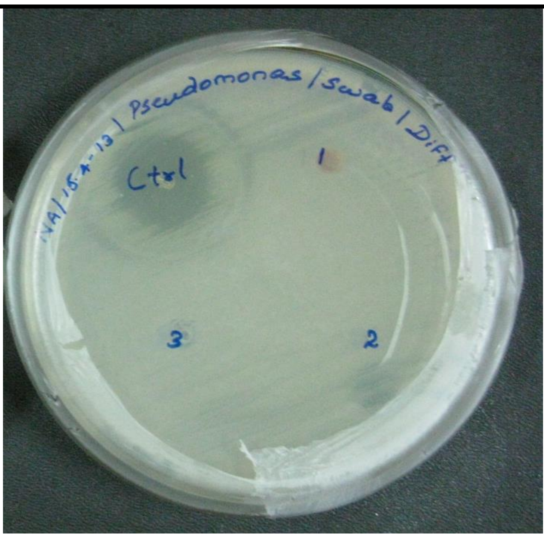

Fig. 5a Bioassay on *P. putida*

Fig. 5b Bioassay on *S. mutans*

Fig. 5c Bioassay on *E.coli*

Fig. 5d Bioassay on microbs isolated from soil

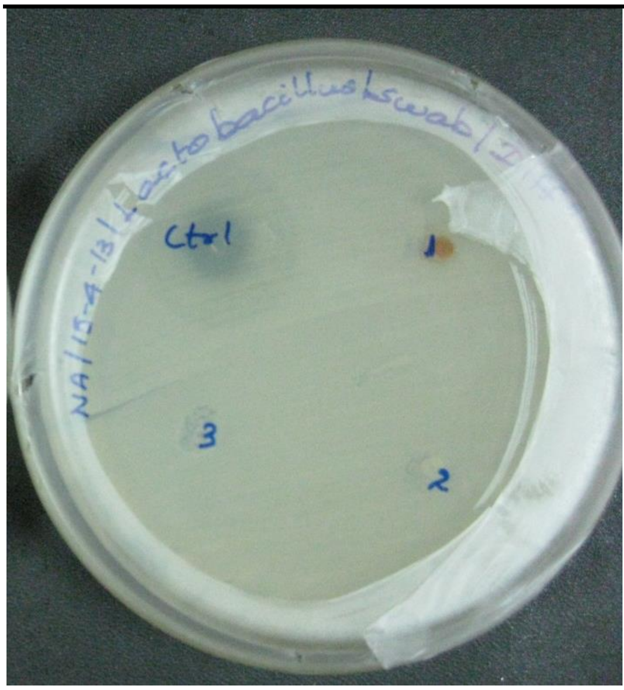

Fig. 5e Bioassay on *L. acidophilus*

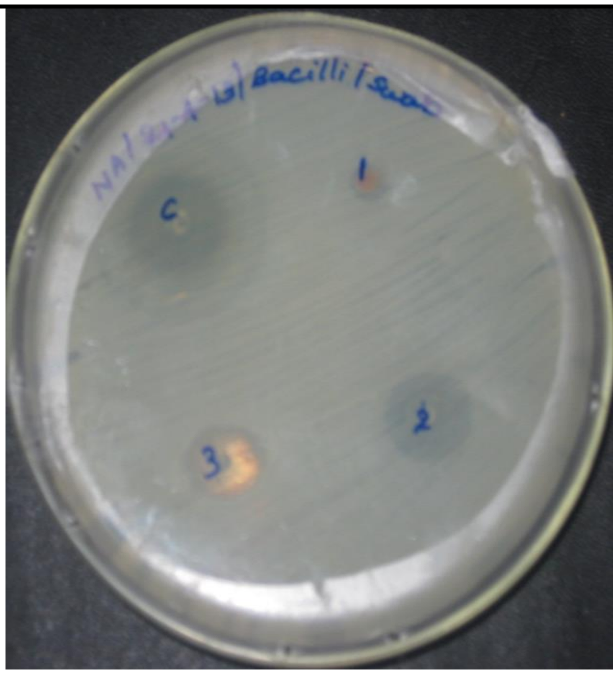

Fig. 5f Bioassay on *Bacilli* sp.

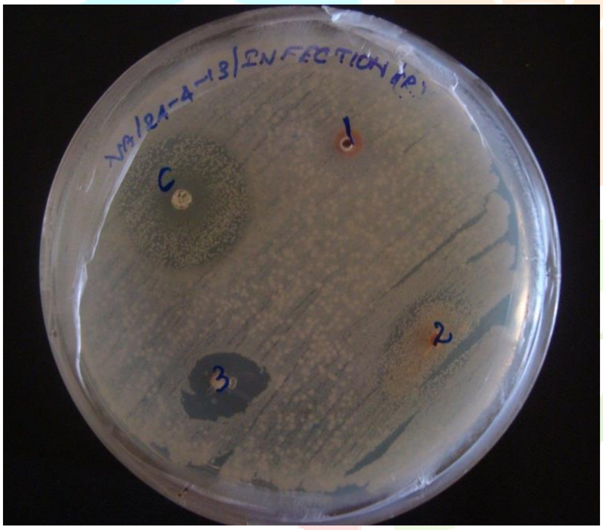

Fig. 5g Bioassay on isolate from blister

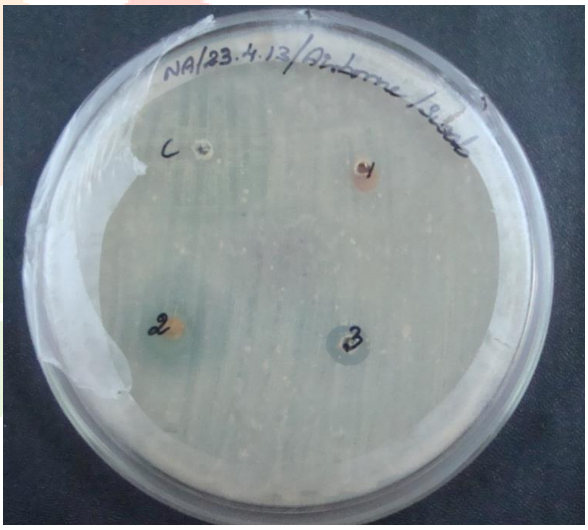

Fig. 5h Bioassay on isolate from mold infection

CONCLUSION

The termitarium sample have good antibiotic and various clinical properties through soil contaminated with certain metabolites which symbiotically produced by termites. Also this work indicating that water extract of termitarium is recommended for *Pseudomonas*, *Bacilli* and blister infection. Petroleum ether extract is recommended for *Streptococcus* and pathogens from soil origin. Ethyl acetate extract is recommended for *E. coli* and mold infection.

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