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THE SCREENING OF ANTIMICROBIAL ACTION OF CELLULOLYTIC FUNGI AND BACTERIA FROM TERMITESGUT AND TERMITES MOUND

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ABSTRACT

Termites are important in the Ecological Ecosystem. They consume and recycle wood, leaf and dung. Termites are usually small or medium sized whitish insect. In this study four different bacteria and seven different fungi isolates were obtained from the gut of the termites. These strains were grown on Carboxymethyl Cellulose Agar medium. One bacteria and one fungus showed significant cellulolytic activity identified by a Congo red assay which gives clear zone. According to the Biochemical tests and sequencing analysis, these isolates were identified as bacteria – *Bacillus tropicus* and fungi – *Malassezia globosa*. The bacteria *Bacillus tropicus* has antimicrobial property against clinical sample and the fungus *Malassezia globosa* has antimicrobial property against clinical sample and antifungal property against plant pathogenic fungi *Clonostachy bysicola*. The fungus *Malazessia globosa* isolates from termites is similar to the fungus that isolated from the termite mound.

1. INTRODUCTION

Termites are important in the ecological ecosystem as they consume and recycle wood leaf and dung and release the nutrients back to the ecosystem .Termites tunnelling help in soil fertility and improvement in soil composition .Termites all live in colonies with reproductive [kings ,queens and nymphs] Soldiers and helpers termites morphological and anatomical adaptations are caste-specific Termites [or white ants] are highly developed social insects which comprise the order Isoptera withinthis order.

There are six families which five are classified as the lower termites [Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae and Serritermitidae]. The lower termites have unique genera species of oxymonad, trichomonad and hypermastigote flagellate. Which are capable of ingesting wood and living as symbionts in the paunch of the hind gut. Most termites are live in tropical, subtropical and warmer temperature zone of the world and subsite on a diet rich in cellulose. they feed on cellulose[R.W.O Brien and M Slaytor et al….2006]

Main character of Termites are usually small or medium sized whitish or colourless insects they have strong biting mouthparts with which to chew seeds wood or leaves. The relationship between the termites and the microbes is a mutualism because both species benefit from the relationship

The digestive system of termites consists of the forgut [consisting of the crop and gizzard] the midgut and hindgut. The hindgut maybe divided into five Successive segments the first proctodeal segment, the second segment called the enteric valve, which controls the entrance into the third segment known as the paunch and in which symbiotic microorganism are abundant. The last two segments are the colon and rectum.

Scientific Classification	
Kingdom	**Animalia**
Phylum	**Arthropoda**
class	**Insecta**
cohort	**Polyneoptera**
superorder	**Dictyoptera**
order	**Blattodea**
infraorder	**Isoptera**

Termites are a delicacy in the diet of some human cultures and are used in many traditional medicines. Several hundred species are economically significant as pest that can cause serious damage to building crops or plantation forests.

The structure of the mounds can be very complicated. Inside the mound in an extensive system of tunnels and conducts that serves as a ventilation system for the underground nest. In order to very good ventilation mound building termites are a group of termite species that live in mound. The mounds sometimes have a diameter of 30 meters. Most of the mounds are in well drained area.

The extensive system of tunnels and conducts have long been considered to help control climate inside the mound. The termite mound is able to regulate temperature and humidity and respiratory gas distribution. The density of air near the surface rises due to heat exchange and is forced below the nest and eventually through the nest again. The purpose of Termite mound function as a ventilation system. A temperature change will cause internal flows in the mound, which move pheromone -like cues around, triggering building behaviour individual termites.

The mound is constructed out of a mixture of soil, termites mound appears solid, the structure is incredibly porous. Its walls are filled with tiny holes that follow outside air to enter and permeate theentire structure.

These mounds are built by the termites themselves using sand, Salida, focal matter and other substances and forming this paste into the familiar herd structure of the termite mound. Vegetation on Termites mounds usually differs highly from vegetation in the surrounding.

The mound soil is generally more fertile than other soil on top of that mound soils have been found to contain more water than their Surrounding, a clear advantage for plant growth. The high tree densities on Termites mounds attract high densities of browsing herbivores due to the high nutrient content in foil age from trees growing on mounds, or perhaps due to the high quantities of food and shelter on mounds.

The mound Represent a specific-habitats for soil micro-organisms. Since the physical properties [water holding capacity, bulk density, structural stability]and the chemical properties [cation exchange capacity, organic matter content and quality] are very different from those of the surrounding soil.
Termite mound soil and normal soil are different in their properties. termites affect the ability of soil support microbes. The influence of termite on soil microbes were determined by microbial analysis

Mound surface appears to be solid and impermeable but it is actually quite porous. The porosity arises from the method that termites employ to grow the mound.

The mound grows by Termites transporting soil on to the mound surface and depositing is there. To get to the surface, termites dig numerous egress tunnels. The mound there for has a dynamic surface. As soil is eroded from the action of wind and rain it is replaced by the action of termites depositing fresh soil. This makes mound structure malleable. The turnover of soil in the mound is seasonal, occurring solely during the rainy season.

The mounds are also often built around trees. This may be due to a shelter effect. The structural form of termite mound provides multiple function. Such as protection from predators and storage of food material. Mound is to provide a homeostatic environment for the nest. The internal and external topologies of the mounds structure facilitate the necessary mechanical functions. Temperature is one of the most significant environmental characteristics which can place strain on the survival of an organism.

2. AIM AND OBJECTIVES

AIM:

This study aims to investigate the microbial diversity in termites gut and termites mounds with following consideration

OBJECTIVES:

1. Isolation of bacteria and fungi from termites mounds and termites gut.

2. Comparison of microbial diversity of termites gut and termites mounds

3. Check the cellulolytic and antimicrobial activity of bacterial and fungal isolates

4. Molecular identification of relevant isolates

3. REVIEW OF LITERATURE

Odelson & Brezank(1983) researched on termites and came to the point that termites have capacity to subsist solely by consuming wood. This is only because their gut contains microbial population that helps in the degradation of lignocellulose in to acetate, the key nutrient of termite metabolism. Within these microbial populations are bacteria, methanogenic. Archea and eukaryotic protozoa.

Kudeo et al(1998), Schmit wagner(2003), Salmassi & Leadbetter(2003) explored the species composition in termite guts and the key genes involved in various biochemical process using molecular techniques, these techniques are based on the extraction and purification of nucleic acidfrom the termite gut.

M Harry Jusseaume,B.Gambier(2001) They conducted a study and tested the use of the RAPD(Random Amplified polymorphic DNA) moleculy markers as a way to estimate the similarity of the microbial communities in various termite mounds and soils in tropical ecosystem, termites activities induce change in the chemical and physical properties of soil. Successful 16SrDNA amplification provided evidence of the occurrence of bacterial DNA in termite construction includingboth soil feeder and fungus grower material.

Saliou fall,Jerome hemalin(2007) conducted a study to characterize the specificity of bacterial communications within mounds with respect to the digey and soil orgnis of the moynds. DGGE Analysis revealed a drasty difference between the genetic structure of the bacterial communicaties of the termite gut and the mound, soil feeding termite mound was dominated by the Actinobacteria phylum, where as the firmicutes and proteobacteria phyla dominate the gut section of termites

Michae E Scharf,Aurelien Tartar(2008) Lignocellulose is a nutritionally poor food source that is highly resistant to enteric degradation. Termite however have the unique ability to digest lignocellulose often using it as a sole food source. Termites have a symbiotic association with prokaryotic and eukaryotic guts symbionts. Termite digeistome can be defined as the pool of genes, both termites and symbiont that contribute to lignocellulose depolymerisation and digestion as well as simple sugar fermentation nutrient transport and nutrient assimilation

Jual A Bonachela, Robert M Pringle(2015)Self organized spatial vegetation patterning is widespread and has been described using models of scale dependent feedback between plants and water on homogeneous substrate. Heterogeneity by altering soil properties, there by enhancing plant growth.
Rather, mound filed landscape are more robust to aridity, suggesting that termite may help stabilize ecosystem under global change.

Pourramzan,GR Ghezelbash(2012) The aim of the study was to isolate and characterize the cellulose degrading bacteria from the gut of the local termite, Microceroterms diversus (Silvestri),inhibiting the khuzestanbprovince of iran the result of the study showed that three cellulose degrading bacteria

isolated from the local termite guts belonged to the genera Acinectobacter, pseudomonae and staphylococcus and four cellulose degrading bacteria belonged to enterobacteriacea and bacillaceafamilies.

Vera Tai, Erick R James (2015) discovered about the distinct community of archaea, bacteria and protists found in the hind guts of lower termites. They analysed the microbial composition of parabasalids and bacteria in the hindguts of lower termites by pyrosquencing variable regions of the small-subunits of rRNA gene. The composition of the parabasalids communities was found to be strongly structured by the phylogeny of their hosts.

Huxley M Makonde, Romano Mwirichia(2015), in this study they compared the bacterial diversity and community structure between termite gut, their mounds and surrounding soil by analysing 16S rRNA gene sequences. A wood header termite, three fungus cultivating termites and their associated mounds were analysed. The results showed significant difference in bacterial community compositions and strucy between the gut and corresponding soil samples.

Saliou fall, Jerome Hamelin, Farma Ndiye(2007).the aim of their study was to characterize the specificity of bacterial communities within mounds with respect to the digestive and soil origins of the mounds. DGGE Analysis revealed a drastic difference between the generic structure of the bacterial communities of the termite gut and the mound. Soul feeding termite gut was dominated by the Actinobacteria phylum, whereas firmicutes and Proteobacteria phyla dominate the gut section of the termite and the surrounding soil.

Ben Jesuorsemwen Enagbonma,Bukola Rhoda Aremu(2019),in this study they aim to access the bacterial functional diversity in termite mound soils with assumption that significant differences will be observed in the functional diversity of bacteria between the termite mound soils and their surroundings soils and that each environment has a distinguishing metabolic profile. Their results revealed that the relative abundance of 16 functional categories differed significantly between both habitats. The alpha diversity and beta diversity analysis showed difference. The variation in the soil physical and chemical properties existing between the two environments were held accountable for thedifference in bacterial functional structure.

Ben Jesuorsemwen Enay, Carolina Fadeke Ajilogba(2020), This study focused on analysis of the compositional and diversity of bacteria in termite mound soil in comparison with the surrounding soils

to verify the assertion that the high nutrient concentration in termite mound soil influence a complex

diversity of microorganisms. The study showed that both the environment have several soil bacterial phyla in common. Diversity analysis showed that bacterial composition was different among the four sites. The study also revealed a lot of unclassified groups of bacteria of this could point to presence of potentially novel species.

Carlos m Aguero, Pierre-Andre Eyer(2021), Termites are intimately tied to the microbial world, as they utilize their gut microbiome for the conversion of the plant cellulose in to necessary nutrients. They characterized the bacterial and fungal communities in the surrounding soil in the nest galleries, and on the cuticle of workers. They found that the galleries provide a more beneficial microbial community than the surrounding soil. Bacterial and fungal diversity was highest in the soil, lower in the galleries, and least on the cuticle. Bacterial communities clustered together according to the substrate from which they were sampled, but this clustering was less clear in fungal communities.

Qing-lin-chen,hang-wei-hu(2021) conducted a survey of 134 termite mounds across 1500 km in northern Australia and found that termite mounds significantly differed from bulk souls in the microbial diversity and community compositions.

4. MATERIALS AND METHODS

4.1. Collection of termite and termite mound

Termite and termite mound were collected from KFRI-Garden Peechi, by using sterile forceps, and screw cap bottle. the photographs of the termite and termite mound were taken by mobile camera at the time of the collection. The collected time of the collection. The collected termite sample were kept in screw cap bottle. Remove the cap and cover the bottle with a sterile cotton cloth to create a suitable environment for termite survival.

4.2. Dissection and isolation of termite<u>In Termite</u>

- Take a drop of sterile distilled water on a sterile clean glass slide
- Use sterile forces to take termite from screw cap bottle filled with termite mound
- Then the medium sized suitable termite is placed on a drop of water to a glass slide
- Take the gut region using a sterile needle
- Wash the gut with ice cold Nacl [0.1%]
- Gut is chopped with a sterile blade then the sample is transferred into sterile Eppendorf tube
- Add about 0.3ml buffer
- Homogenize by using votex with 2 min
- Homogenized sample is placed on a centrifuge for centrifugation 10000 rpm for 2 min at 35^0c
- After centrifugation take pellet and remove supernatant.

4.3. Serial dilution and spread plates

- After centrifugation 1 ml pellet was taken for serial dilution
- Serial dilution was done with 1 ml Eppendorf tube
- 0.1ml of sample from dilution [10^{-1} to 10^{-6}] was transferred into each petri-plate containing nutrient agar for bacteria and PDA for fungi

- Incubate the nutrient agar plates for 24hours at 37^0 c
- Incubate PDA plates for 14 days at room temperature
- After incubation growth were observed in each place of NA and PDA.

In Termite mound

- Take 1 gm of mound sample from screw cap bottle that filled with mound
- Pour 9ml of distilled water into the sterile test tube containing 1 gm of soil sample
- Mix thoroughly with the help of sterile glass pipette
- Serial dilution was done with the help of 1 ml glass pipette [$10^{-1}$to 10^{-6}]
- 0.1 ml of sample from dilution $10^{-1}$to 10^{-6} was transferred into each petri-plate containing nutrient agar for bacteria and PDA for fungi
- Incubate the nutrient agar plate for 24 hrs at 31^0c
- Incubate PDA plates for 14 days at room temperature
- After incubation growth are observed in each plates of NA and PDA

4.4. Colony identification, morphology observation of bacteria

- After incubation each different individual colonies were spotted on NA plates and quarter streaking was performed using another set of plates
- Similarly, the individual colonies in the PDA plates were inoculated on another set of PDA plates
- There after colony taken from plates and performed gram staining

4.4.a) Gram Staining

- Loopful of culture were taken on a clean glass slide with 1 drop of sterile distilled water
- Mix thoroughly and prepare smea
- Heat fixation
- Add 1 drop of crystal violet. After 1 min wash the crystal violet under tap water
- Later add 2 to 3 drops of grams iodine for 1 min
- Wash the grams iodine under tap water after 1 min
- Add 95% ethyl alcohol [decolouriser] for 30 sec
- Wash the decolouring agent under tap water
- Air dry
- Observe it under 45X then 100x in oil immersion

- Biochemical methods are performed for the identification bacteria by using the colonies from NA plates

4.5. colony morphology and microscopic of fungus

- Individual colonies were observed on the PDA plates after incubation at room temperature for 7-14 days
- Similarly, the individual colony in the PDA plates were inoculated on another set of PDA plates
- There after colonies are taken from PDA plates and perform slide culture techniques

4.5a) Slide culture

- Aseptically with a pair of forceps place a sheet of sterile filter paper in a petri-dish
- Place a sterile U- shaped glass rod on the filter paper [rod is sterilised by flaming it held of forceps]
- Pour enough sterile water [about 4 ml]
- On filter paper to completely moisten it
- With forceps place a sterile slide on the U- shaped rod
- Gently flame a scalpel to sterilize and cut a 5 mm square block of the medium from the place of Sabouraud's-Dextrose-Agar (SDA)
- Pick up the block of the ager by inserting the scalpel and carefully transfer this block aseptically to the centre of the slide
- Inoculate four slides of agar square with spores or mycelial fragments of the fungus to be examined
- Aseptically place a sterile cover glass on the upper surface of the agar cube
- Place the cover on the petri-dish and incubate at room temp for 48 hrs
- After 48 hrs examine the slide under low power

4.6.a) Bacteria <u>Antibacterial property</u>

For this identification, collect clinical samples such staphylococcus aureus, klebsiella, pseudomonas,E.coli,

- Take nutrient agar plates and inoculate the isolated bacteria from gut and mound
- After inoculation incubate 24-48hrs at 37^0c
- After the incubation growth will appear on NA plates
- For dual culture method make a central line on a petri-plate and inoculate the clinical

sample opposite to the growing bacteria from the gut and mound

- Continue the incubation at 37^0c for 7 days
- Similarly control plates are maintain to compare the growth of bacteria that is inoculated against the clinical sample
- Incubate the control plates

After incubation measure the diameter in both control and dual culture plate at 2,5&7thday

Antifungal property

- Take the nutrient agar plates and inoculates the bacteria from gut and mound
- After the inoculation, incubate the NA plates for 24 hrs at 37^0c
- After the incubation growth will appear on Na plates
- For the dual culture make a central line on petri-plate and inoculated the plant pathogenic fungi [clonostachy bysicola] to the growing bacteria from the gut andmound
- The Na plates is kept at room temperature
- Similarly control plates are maintain to compare the growth of bacteria that is inoculated against plant pathogenic fungi [clonostachys hyssicola]
- Incubates the control plates
- After incubation measure the diameter in dual culture plate and compare it with the control plate

4.6 b) Fungus Antibacterial property

- Take a PDA plate and inoculate the fungus from gut and mound
- After the inoculation incubate the PDA for 24-48hrs at room temperature
- For the duel culture make a central line on a petri-plate and inoculate the clinical sample opposite to the growing fungus from gut and mound
- The PDA plates were kept at room temperature for 7-14 days
- Similarly control plates are compared the growth of fungus that inoculate against the clinical sample
- Incubate the control plates
- After incubation measure the diameter in both control and dual culture plates at 2,5&7 days

Antifungal property

- Take a PDA plates and inoculate the fungus from gut and mound
- After the inoculation incubate the PDA for 24-48hrs at room temperature
- For the dual culture make a central line on a petri-plate and the plant pathogenic fungi [clonostachy bysicola] opposite to the growing fungus from gut and mound
- The PDA plates were kept at room temperature for 7-14 days
- Similarly control plates are compared the growth of fungus that inoculate against the plant pathogenic fungi [clonostachy bysicola]
- Incubate the control plates

After incubation measure the diameter in both control and dual culture plates at 2,5, &7days

4.7 Cellulolytic activity of gut and mound bacteria

- Inoculate the bacteria in CMC media [Carboxy methyl cellulose agar] for 3 days and incubate at 37^0c
- At the 4^{th}day perform sterile plate in CMC media to obtained pure colonies
- Incubate for 2 days at 37^0c
- Flood the plate with .1%congored for 20 min
- Wash with 1m Nacl for 20 min
- Observe the zone formation

4.8 Biochemical test for identification of bacteria1. methylene red test

Principle

- Used to determine the ability of organism to produce stable acid as the end product of glucose fermentation
- If the large amount of acid that include formic acid acetic acid and lactic acid succinic acid from glucose fermentation the colour of both will remain red after the addiction of methyl red indicator(positive)
- The organism do not produce acid, the both medium will changes to yellow indicating a negative test

Procedure

- Pure culture organism is inoculated in the MRVP broth

- Incubate at 37^0c 4 hrs
- Add 5 to 6 drop of methyl red indicator
- Observe the colour change of broth medium

2. Voges-Proskauer test

Principle

- The VP test is used to determine if an organism produce acetyl methyl carbinol is converted to diacetyl in the presence of ∞ naphthol, strong alkaline [45% KOH] and atmospheric oxygen

Procedure

- Inoculate MRVP broth with a pure culture of organism
- Incubate at 35-37^0c for a min of 48 hrs in ambient air
- Add 6 drops of VP reagent [∞ Naphthol] and 2 drops VP reagent II [40% KOH]
- Observe for the colour change in the both mediu

3. Citrate test

Principle

- Citrate agar is used to test an organism's ability to utilize citrate as a source of energy
- The medium contains citrate as the sole carbon source and inorganic ammonium salt [as] the sole source of nitrogen
- Bacteria that can grow on this medium produce an enzyme citrate-permease capable of converting citrate to pyruvate
- When the bacteria metabolize citrate the ammonium salts are broken down to ammonia which increases alkalinity
- The shift in PH turns the bromothymol blue indicator in the medium from green to blue above PH 7.6

Procedure

- Sterile the slant back and forth with a light inoculum picked from the centre of a well – isolated colony
- Incubate aerobically at 35 to 37 ^{0}c for up to 4-7 days

- Observe a colour change from green to blue along the slant\

4. Urease test

Principle

- The urease test identifies those organisms that are capable of hydrolysing area to produce ammonia and carbon dioxide
- It is primarily used to distinguish urease – positive protea from other Enterobacteriaceae

Procedure

- Sterile the surface of a urea ager slant with a portion of a well -isolated colony
- Leave cap on loosely
- Incubate the tube at 35-37^{0}c in ambient air for 48 hrs to 7 days
- Examine for the development of a pink colour for a long as 7 days

5. catalytic test

Principle

- The enzyme catalase mediates the break down of hydrogen peroxide into oxygen and water
- The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide and rapid elaboration of oxygen bubbles occurs
- The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hrs old

$2H_2o_2$---------- $>2H_2o+o_2$[gas bubbles]

catalase

procedure [slid method]

- Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean dry glass slide
- Place a drop of 3% H_2O in the glass slide
- Observe for the evolution of oxygen bubbles

6. oxidase test

Principle

- The oxidase test is used to determine if an organism possess the cytochrome C oxidase enzyme
- The test is used to differentiate pseudomonas from related species
- The test can also be performed by flooding the culture plate with oxidase reagent but is not recommended because the reagent kills the bacteria
- The test is spot method based on colour change and useful in the initial characterisation of gram -negative micro-organism
- It is used to differentiate oxidase positive micro -organisms such as Aeromonas SP. Pseudomonas SPP and Halophila SP. From the oxidase negative Enterobacteriaceae

Procedure

- Take a commercially available oxidase disc containing the reagent
- Pick the isolated colony to be tested and rub it in the disc
- Observe for colour change within 10 second

5.RESULTS AND DISCUSSION

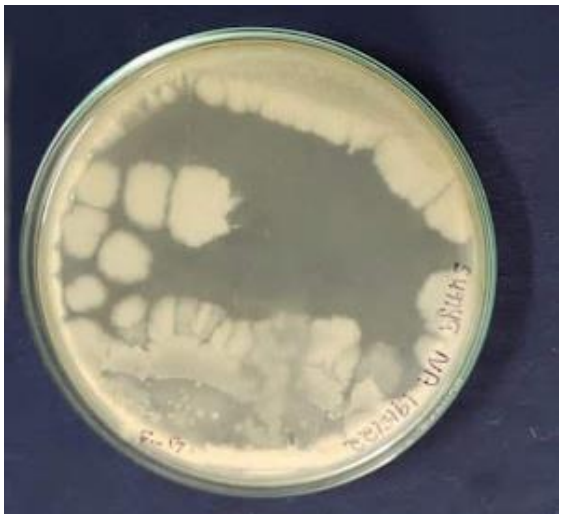
Isolation And Identification of Bacteria from Termite Gut

Four bacteria are isolated from termite gut. The bacterial isolates were obtained by dilution plate method at different dilution 10^{-3}, 10^{-4} and 10^{-5} plating on NA medium [fig 1].

Fig 1:

A

B



C

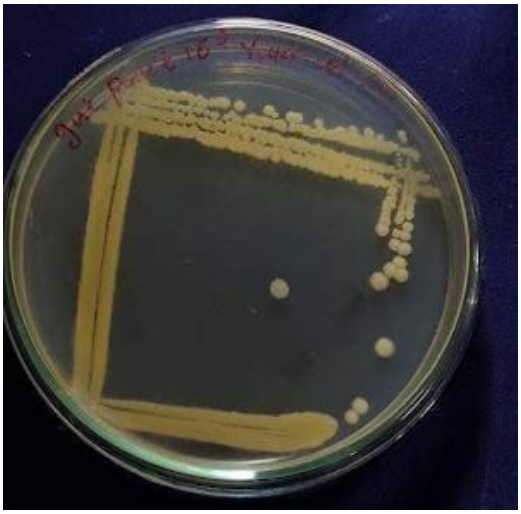
Morphology And Microscopic Observation

Morphologically all the bacterial isolates exhibited typically colonization characters. These bacteria produced white colonies and some were yellow, orangecolour colonies on nutrient agar medium within 2 to 3 days of incubation.

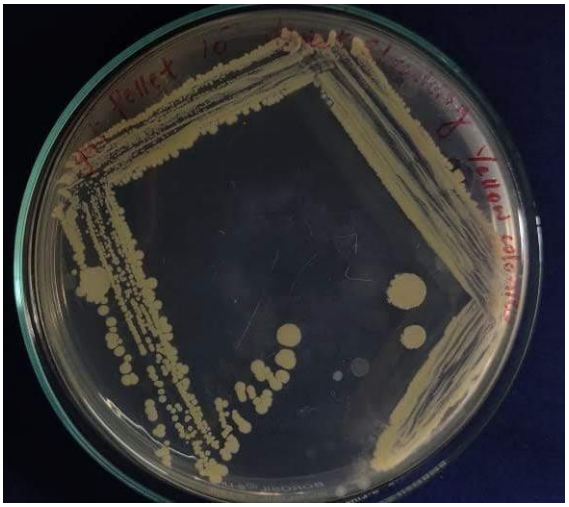
Table 1: colony characters of the individual isolates

Test	AB 1	AB 2	AB 3	AB 4
Size	Medium	Small	Medium	Large
Shape	Round	Round	Round	Mucoid
Margin	Entire	Entire	Entire	Irregular
Elevation	Raised	Raised	Raised	Flat
Surface tension	Smooth	Smooth	Smooth	Glisten
Optical character	Translucent	Translucent	Translucent	Opaque
Pigmentation	Orange	Yellow	White	Creamy

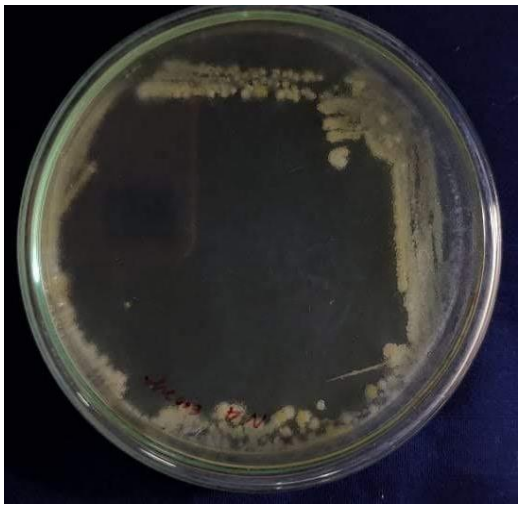
Fig 2:



AB 1



AB 2

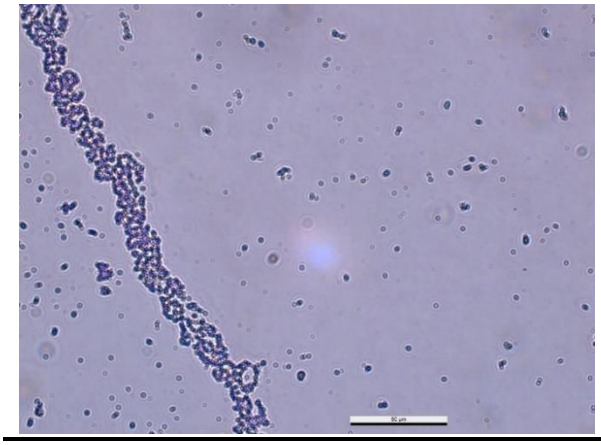


AB 3

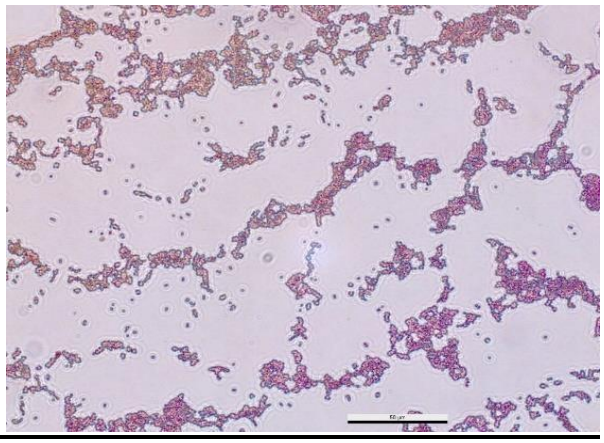
AB 4

Table 2: Gram stain reaction of Bacterial Isolation

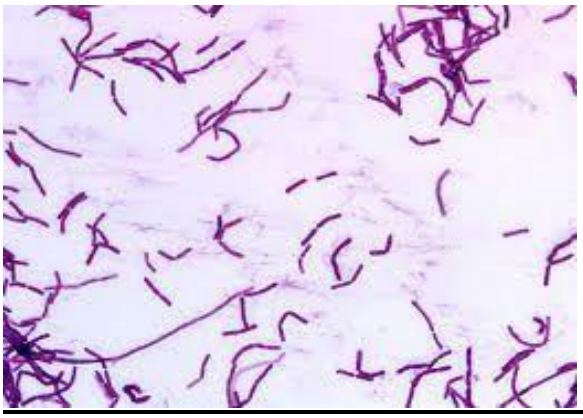
Bacterial Isolates	Cell shape	Gram reaction
AB 1	Cocci	Positive
AB 2	Cocci	Negative
AB 3	Cocci	Negative
AB 4	Filamentous	Positive



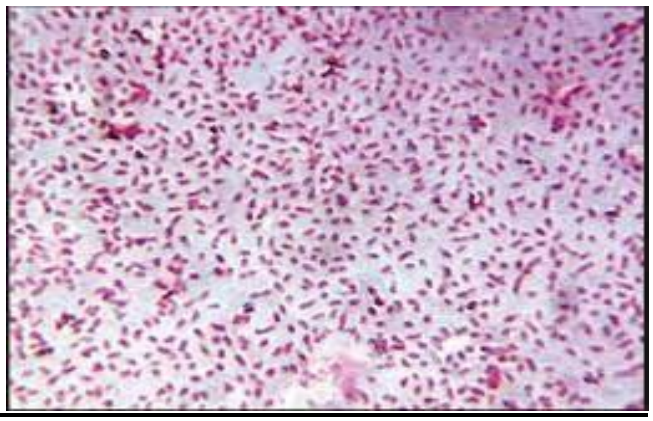
AB1



AB3



AB4



AB2

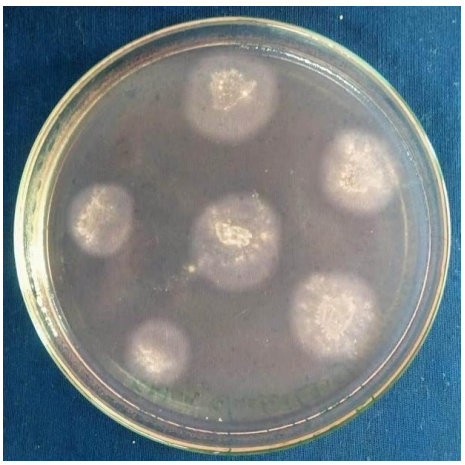
Cellulolytic Activity

Bacterial isolates were screened for cellulolytic activity using Congo red agar plates. Clear zone formation was observed. Confirm the ability of bacteria to produce cellulase enzyme. Among the bacteria one of them [AB 4] isolates is found to be positive by forming colour zone.

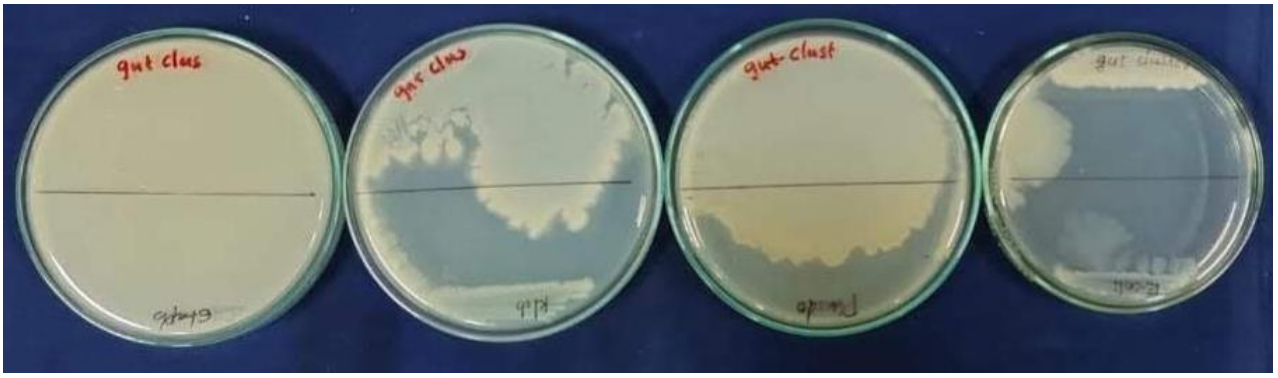
Table 3:

Sl no.	Sample isolates	No. of colour zone
1	AB 4	6

Fig 3:



Determination of antibacterial and antifungal property of gut bacteria



Cellulolytic activity showing the particular sample [AB 4] were dual cultured to check if they have any antibacterial and antifungal property.As a result, the bacteria showing antibacterial property against clinicalsample such as klebs, psuedo and staph

Control:

Kleb

Pseudo

Staph

E.coli

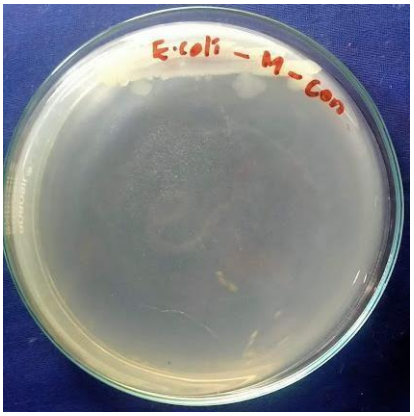


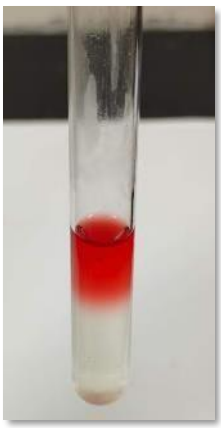
Table 4:

Sample	Days of incubation	Control clinical sample	Dual culture			
			Gut bacteria	(mm)	Clinical sample	(mm)
AB 4	2 days	Psuedo -20mm Kleb –30 mm Staph -30 mm E.coli -20 mm	AB4	40 mm 42 mm 40 mm 20 mm	Pseudo Kleb Staph E.coli	2 mm 10 mm 5 mm 5 mm
	4 days	Psuedo -40 mm Kleb –40 mm Staph -40 mm E.coli -20 mm	AB4	71 mm 73 mm 80 mm 40 mm	Pseudo Kleb Staph E.coli	5 mm 25 mm 5 mm 6 mm
	5 days	Psuedo -60 mm Kleb –60 mm Staph -60 mm E.coli -47 mm	AB4	73 mm 73 mm 85 mm 40 mm	Pseudo Kleb Staph E.coli	10 mm 34 mm 5 mm 10 mm

Table 5: Biochemical tests conducted for bacterial isolates [AB-4]

Isolated strain	AB-4
MR	+ve
VP	+ve
Citrate	-ve
Urease	+ve
Catalase	+ve
Oxidase	+ve
Nitrate	-ve

Fig 5:



MR +ve

VP +ve

Citrate -ve

Urease -ve

Oxidase -ve

Nitrate -ve

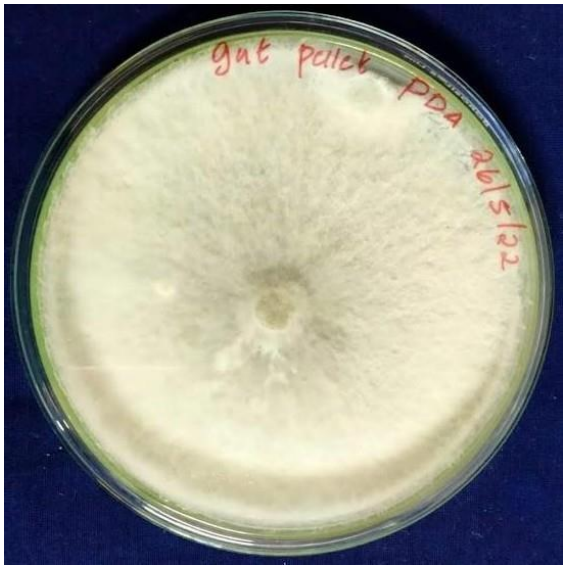
Isolation and Identification of Fungus from Termite Gut

Seven fungi are isolated from the termite gut, the fungus isolates were obtained by dilution plate method at different dilution 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} plated on PDA plates.[fig 6]

Microscopic Observation of Fungus

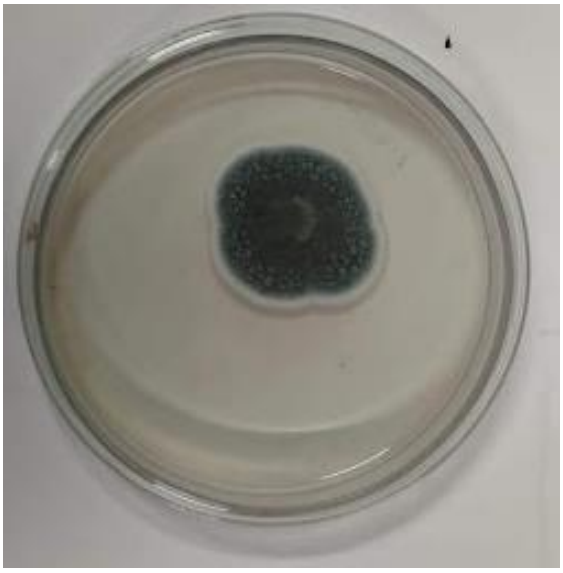
By using slide culture technique, microscopically all the isolates exhibit typical spores or mycelial growth. Most of the isolates have well developed spores on PDA plates within 6 to 7days of incubation. [fig 7]

Fig 6:



A

B



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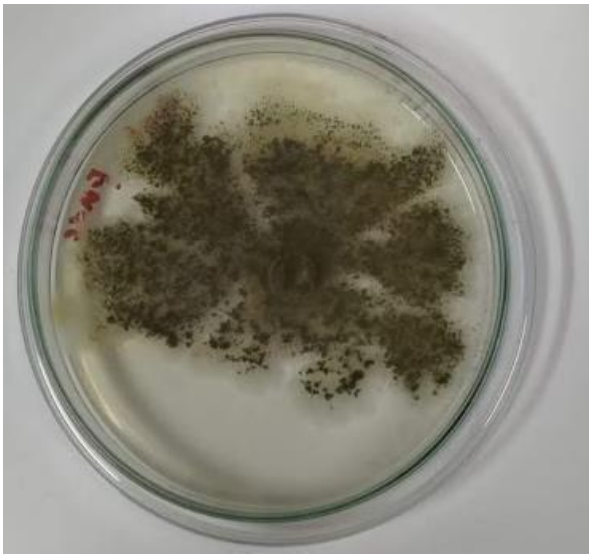
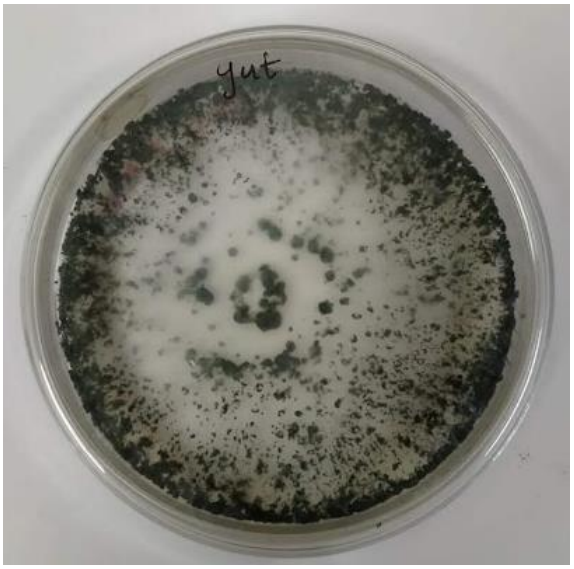


D

E



F

G

Determination of antibacterial and antifungal property of gut fungus

By using dual culture techniques to check if they have any antibacterial, antifungal property and cellulytic activity. As a result [fungus B] shows antifungal property against known plant pathogenic fungus. [clonostachys byssicola] also shows antibacterialproperty against clinical sample such as kleb, pseudo, staph and E.coli.

Fig 7:

Pseudo

Kleb

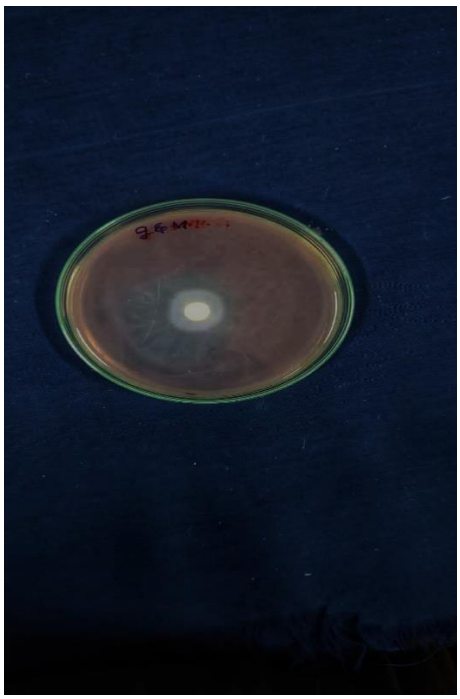
Staph

Fig 7 b:

Antifungal property

Control

Fig 7c



Cellulolytic Activity (Zone Formation)

Isolation and identification of bacteria from termite mound

Only one [AB-5] bacteria were isolated from termite mound. The bacterial isolates wereobtained by dilution plate method at different dilution 10^{-3}, 10^{-4}, and 10^{-5}, plating on NAmedium. Same type of bacteria was isolated fromall these dilutions.

Morphology and microscope observation

Morphologically the bacterial isolates exhibit typical colonization characters, white mucoid colonies. Most of the isolates formed well developed colonies on nutrient agar medium within 2 to 3 days incubation.

Colony characters of the bacteria

Test	AB-5
Size	large
Shape	Mucoid
Margin	Irregular
Elevation	Flat
Surface texture	Glisten
Optical character	Opaque
Pigmentation	Creamy

Table 6

Gram stain reaction of bacteria

Bacterial isolates	Cell shape	Gram reaction
AB5	cocci	negative

Table 7

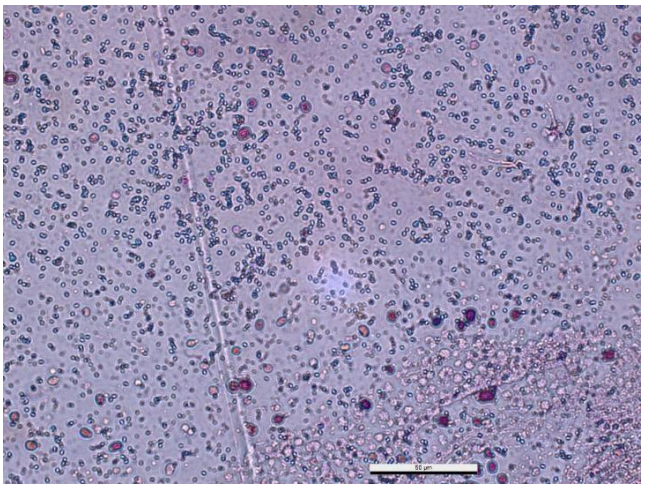


Figure 8

Cellulolytic activity

Bacterial isolates were screened for cellulolytic activity using Congo red agar plates. There isno zone formation. [no cellulolytic activity].

Sl no.	**Sampleisolates**	**Colour zone formationdiameter [mm]**
1	**AB-5**	**0**

Table 8

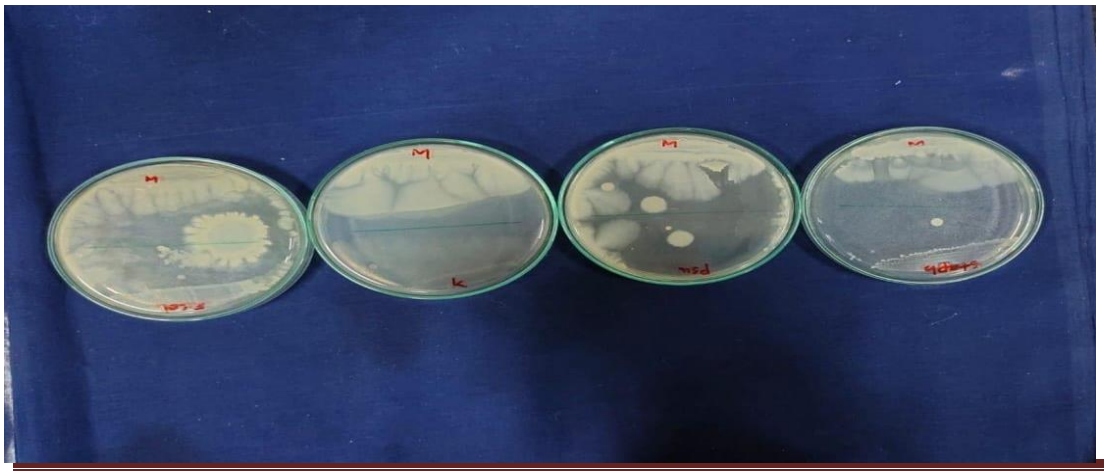
Determination of antibacterial and antifungal property of termitemound bacteria

[AB-5] were dual cultured to check if they have any antibacterial and antifungal property. Asa result, the bacteria showing antibacterial property against clinical sample such as Klebs, Pseudo and Staph. But they have noantifungal property.

Sample	Days of incubation	Control clinical sample	Dual culture			
			Moundbacteria	Diameter [mm]	Clinical sample	Diame [mm]
AB 5	2 days	Pseudo – 20 mm Kleb -30 mm Staph – 30 mm E.coli – 20 mm	AB 5	40 mm 42 mm 40 mm 30 mm	Pseudo Kleb Staph E.coli	10 mm 10 mm 5 mm 5 mm
	4 days	Pseudo – 40 mm Kleb -30 mm Staph – 30 mm E.coli – 20 mm	AB 5	71 mm 73 mm 80 mm 40 mm	Pseudo Kleb Staph E.coli	5 mm 25 mm 5 mm 10 mm
	5 days	Pseudo – 60 mm Kleb -60 mm Staph – 60 mm E.coli – 47 mm	AB 5	73 mm 73 mm 85 mm 40 mm	Pseudo Kleb Staph E.coli	10 mm 34 mm 5 mm 10 mm

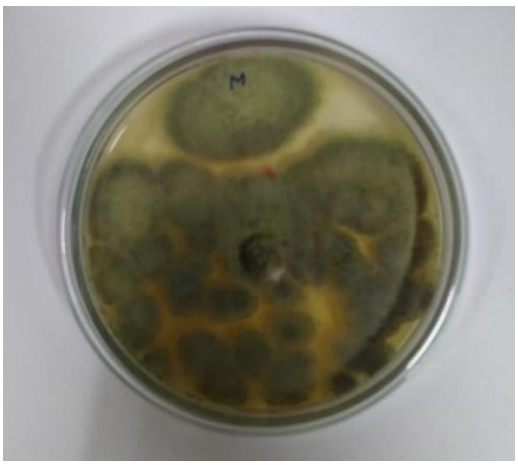
Table 9

Fig (AB5)

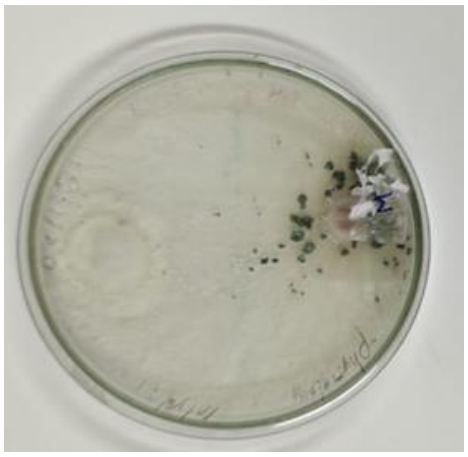


Isolation and identification of fungus from termite mound

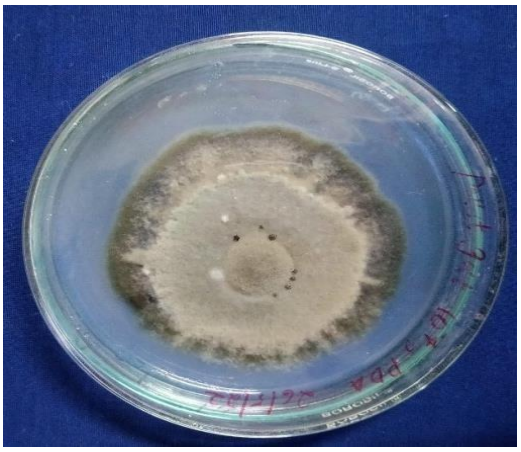
Seven fungi are isolated from the termite mound the fungus isolates were obtained by dilutionplate method at different dilution 10^{-2}, 10^{-3} ,10^{-4} and 10^{-5}plated on PDA plates [figure9]



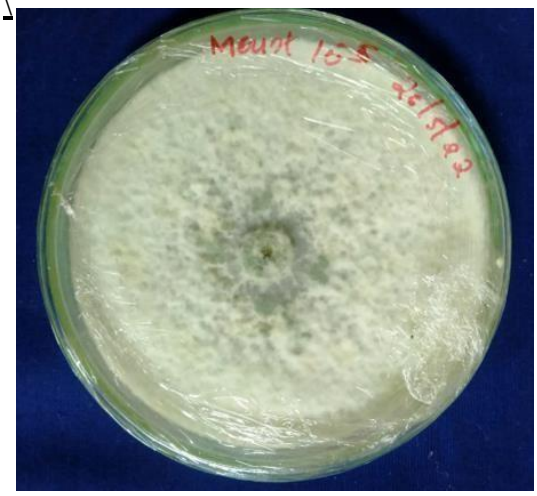
H



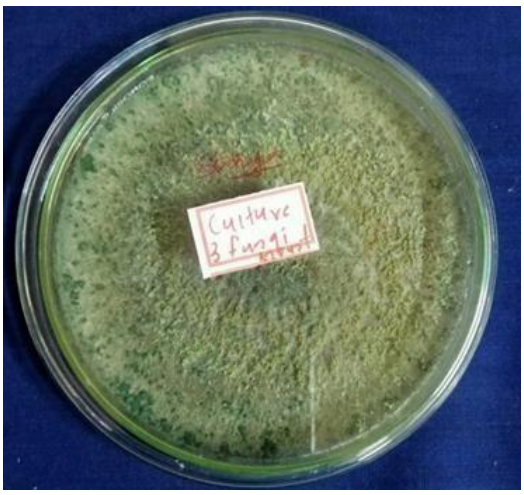
I



J



K



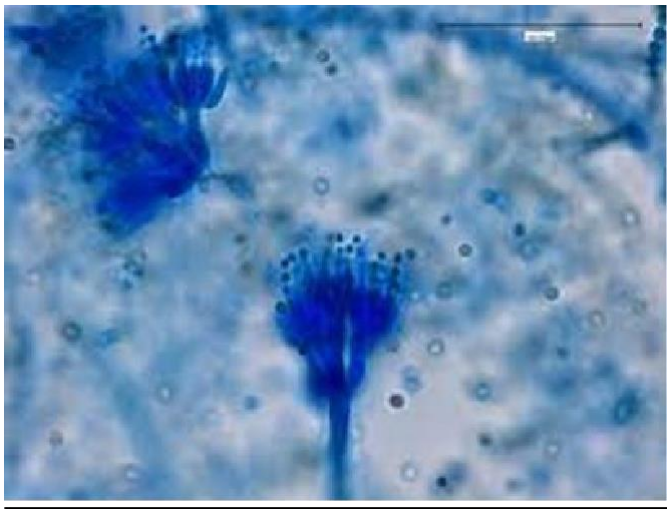
L

M

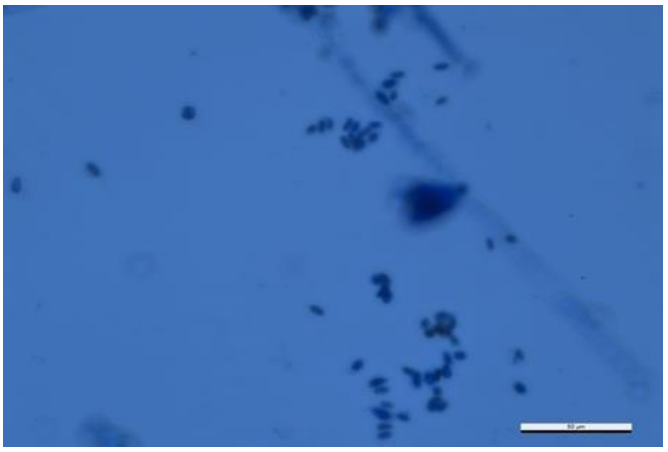
N

Identical fungus from termite gut and mound

From termite gut and mound total 14 species of fungus were observed in which 5 of thefungus were similar in both termite mound and gut.



A



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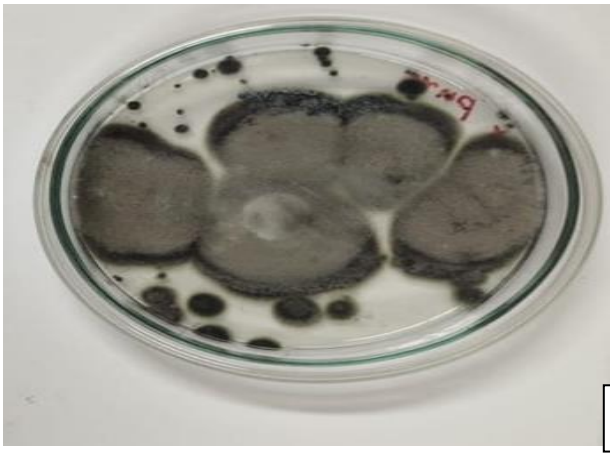

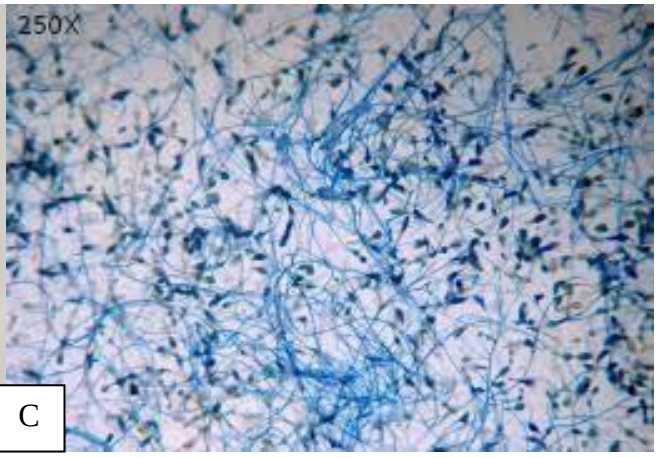

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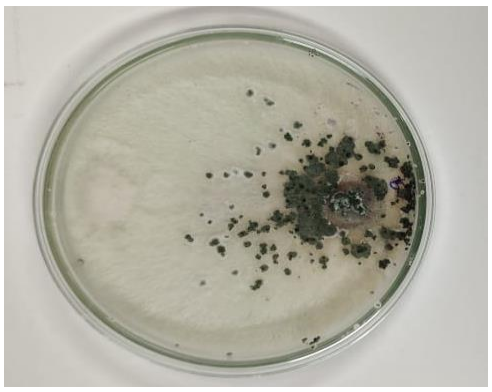
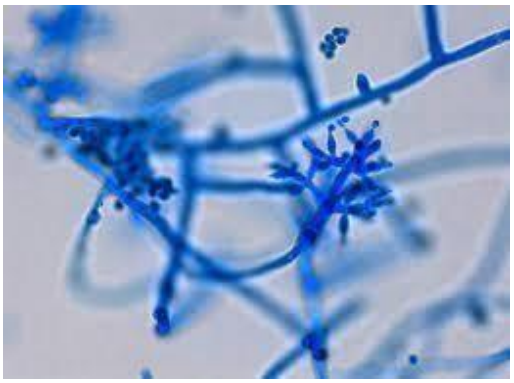
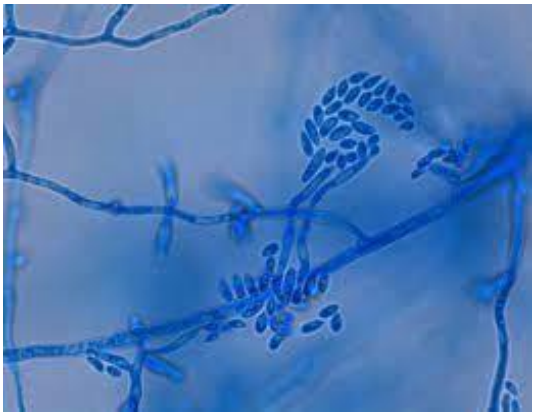




D



E

After slide culture the spores were identified A) Penicillium sp, C) Alternaria sp, D) Trichoderma sp, E) Fusarium sp

COMPARISON STUDY OF TERMITE GUT AND MOUND

The AB4 bacteria [gut bacteria] shows antibacterial and cellulolytic activity. Presently 4 bacteria were observed from the termite gut. Out of which AB4 bacteria shows antibacterial and cellulolytic activity the rest of other bacteria were eliminated and further of focusing molecular identification was done with AB4 bacteria

In case of termite mound the observed bacteria only shows antibacterial property. Cellulolytic activity was absent. As a result of the termite mound bacteria was eliminated.

Fungus

The termite mound and gut 14 species of fungus were observed in which 5 of the fungus were similar in both termite mound and gut. 5 of out of which the fungus B shows antibacterial, antifungal and cellulolytic activity there for further focusing molecular identification was done with the fungus B and the rest of the fungus were eliminated.

MOLECULAR IDENTIFICATIONBACTERIA(AB4)

Result of DNA AnalysisI Protocols

Genomic DNA Isolation from Bacteria

Genomic DNA was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions.

A part of culture is taken in a microcentrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K was added and incubated at 56 o C in a water bath until it was completelylysed. After lysis, 5 µl of RNase A (100 mg/ml) was added and incubated at room temperaturefor 5 minutes. 200 µl of B3 buffer was added and incubated at 70 o C for 10 minutes. 210 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipettedinto NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 xg for 1 minute. The NucleoSpin® Tissue column was transferred to a new 2 ml tube andwashed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer. Afterwashing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was elutedout using 50 µl of BE buffer.

Agarose Gel Electrophoresis for DNA Quality and Quantity check

The quality of the DNA isolated was checked using agarose gel electrophoresis.
1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresiswas performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system(Bio-Rad).

Page 2 of 5 PCR Analysis

2X Phire Master Mix 5µLD/W 4µL

Forward Primer 0.25µLReverse Primer 0.25µL DNA 1µL

Primers used Target Primer

Name Direction Sequence (5’ 3’)16S rRNA

16S-RS-F Forward CAGGCCTAAC□ACATGCAAGTC

16S-RS-R Reverse GGGCGGWGTGTACAAGGC

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profile16S rRNA

95 o C - 5.00 min

95 o C - 30 sec

60 o C - 40 sec 35 cycles

72 o C - 60 sec

72 o C - 7.00 min4 o C - ∞

Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer

containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 4 µl ofPCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA Page 3 of 5 ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).ExoSAP-IT Treatment

ExoSAP-IT (USB) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal ofunwanted primers and dNTPs from a PCR product mixture with no interference in downstreamapplications.

Five micro litres of PCR product is mixed with 0.5µl of ExoSAP-IT and incubated at 37 o C for 15 minutes followed by enzyme inactivation at 85 o C for 5 minutes. Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol.

The Sequencing PCR mix consisted of the following components:D/W 6.6µL

5X Sequencing Buffer 1.9µL Forward Primer 0.3µL Reverse Primer 0.3µL Sequencing Mix 0.2µL Exosap treated PCR product

1µL

SequencingPCR amplification profile96 o C - 2min

96 o C - 30sec

50 o C - 40sec 30 cycles60 o C - 4min

4 o C - ∞

Post Sequencing PCR Clean upPage 4 of 5

D/W 5 µl

3M Sodium Acetate 1 µlEDTA 0.1 µl

100% Ethanol 44 µl

1. Mix D/W, 125mM EDTA, 3M sodium acetate pH 4.6 and ethanol were prepared andwere properly mixed.
2. 50 µl of mix was added to each well in the sequencing plate containing sequencingPCR product.
3. Vortex by Mixmate vortex and Incubated at room temperature for 30 minutes
4. Spun at 3700 rpm for 30 minutes
5. Decanted the supernatant and added50 µl of 70% ethanol to each well
6. Spun at 3700 rpm for 20 minutes.
7. Decanted the supernatant and repeated 70% ethanol wash
8. Decanted the supernatant and air dried the pellet.

The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems) using Sanger DNA sequencing method.

Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010).

Forward Primer

>SR2981-1-RSF1_G05.ab1
GAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG
ATAACTCCGGGAAACCGGGGCTAATA
CCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCA
CTTATGGATGGACCCGCGTCGCATT
AGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAG
AGGGTGATCGGCCACACTGGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGAC
GAAAGTCTGACGGAGCAACGCCGCGT

GAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGC
TAGTTGAATAAGCTGGCACCTTGACG
GTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTATCCGGAATTAT
TGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGC
TCAACCGTGGAGGGTCATTGGAAACT
GGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATG
CGTAGAGATATGGAGGAACACCAGTG
GCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGGA
GCAAACAGGATTAGATACCCTGGTAG
TCCACGCCGTAAACGATGAGTGCTAGTGTTAGAGGGTTTCCGCCCTTTTA

Reverse Primer

>SR2981-1-RSR1_G06.ab1
TTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAG
AACGGTTTTATGAGATTAGCTCCAC
CTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGT
CATAAGGGGCATGATGATTTGACGT
CATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTA
ATGATGGCAACTAAGATCAAGGGTT
GCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCA
TGCACCACCTGTCACTCTGCTCCCGA
AGGAGAAGCCCTATCTCTAGGGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCT
TCGCGTTGCTTCGAATTAAACCACA
TGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC
GTACTCCCCAGGCGGAGTGCTTAA
TGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGT
TTACGGCGTGGACTACCAGGGTATC
TAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAA
GTCGCCTTCGCCACTGGTGTTCCTC
CATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT
CAAGTCTCCCAGTTTCCAATGACC
CTCCACGGGTTGAGCCCGTGGGCTTTCACATCAGACTTAAAGAACCACCCTGCGC
GCGCTTTACGCCCAATAAT

- Nucleotide test sequence of the strain showed 99.74% of homology with bacillustropicus.

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus tropicus strain MCCC 1A01406 16S ribosomal RNA, partial sequence	Bacillus tropicus	1406	1406	99%	0.0	99.74%	1509	NR_157736.1
Bacillus paramycoides strain MCCC 1A04098 16S ribosomal RNA, partial sequence	Bacillus paramycoides	1406	1406	99%	0.0	99.74%	1509	NR_157734.1
Bacillus pacificus strain MCCC 1A06182 16S ribosomal RNA, partial sequence	Bacillus pacificus	1406	1406	99%	0.0	99.74%	1509	NR_157733.1
Bacillus nitratireducens strain MCCC 1A00732 16S ribosomal RNA, partial sequence	Bacillus nitratireducens	1406	1406	99%	0.0	99.74%	1509	NR_157732.1
Bacillus luti strain MCCC 1A00359 16S ribosomal RNA, partial sequence	Bacillus luti	1406	1406	99%	0.0	99.74%	1509	NR_157730.1
Bacillus albus strain MCCC 1A02146 16S ribosomal RNA, partial sequence	Bacillus albus	1406	1406	99%	0.0	99.74%	1509	NR_157729.1
Bacillus paranthracis strain MCCC 1A00395 16S ribosomal RNA, partial sequence	Bacillus paranthracis	1406	1406	99%	0.0	99.74%	1509	NR_157728.1
Bacillus cereus strain IAM 12605 16S ribosomal RNA, partial sequence	Bacillus cereus	1406	1406	99%	0.0	99.74%	1486	NR_115526.1
Bacillus cereus ATCC 14579 16S ribosomal RNA (rrnA), partial sequence	Bacillus cereus ATCC 14579	1406	1406	99%	0.0	99.74%	1512	NR_074540.1
Bacillus cereus strain CCM 2010 16S ribosomal RNA, partial sequence	Bacillus cereus	1406	1406	99%	0.0	99.74%	1535	NR_115714.1
Bacillus cereus ATCC 14579 16S ribosomal RNA, partial sequence	Bacillus cereus ATCC 14579	1406	1406	99%	0.0	99.74%	1482	NR_114582.1
Bacillus cereus strain NBRC 15305 16S ribosomal RNA, partial sequence	Bacillus cereus	1406	1406	99%	0.0	99.74%	1476	NR_112630.1
Bacillus anthracis strain ATCC 14578 16S ribosomal RNA, partial sequence	Bacillus anthracis	1406	1406	99%	0.0	99.74%	1306	NR_041248.1

FUNGUS

Result of DNA Analysis

DNA Barcoding using universal primers of LSU<u>I Protocols</u>

DNA isolation using NucleoSpin® Plant II Kit (Macherey-Nagel)

About 100 mg of the tissue/mycelium is homogenized using liquid nitrogen and the powdered tissue is transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 is added and vortexed for 1 minute. Ten microlitres of RNase A solution is added and inverted to mix. The homogenate is incubated at 65^{o}C for 10 minutes. The lysate is transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 minutes. The flow through liquid is collected and the filter is discarded. Four hundred and fifty microlitres of buffer PC is added and mixed well. The solution is transferred to a Nucleospin Plant II column, centrifuged for 1 minute and the flow through liquid is discarded. Four hundred microlitre buffer PW1 is added to the column, centrifuged at 11000 x g for 1 minute and flow though liquid is discarded. Then 700 µl PW2 is added, centrifuged at 11000 x g and flow through liquid is discarded. Finally 200 µl of PW2 is added and centrifuged at 11000 x g for 2 minutes to dry the silica membrane. The column is transferred to a new 1.7 ml tube and 50 µl of buffer PE is added and incubated

at 65^{o}C for 5 minutes. The column is then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4^{o}C.

Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

PCR Analysis

2X Phire Master Mix	5µL
D/W	4µL
Forward Primer	0.25µL
Reverse Primer	0.25µL
DNA	1µL

Primers used

Target	**Primer Name**	**Direction**	**Sequence (5’ □ 3’)**
LSU	LROR	Forward	ACCCGCTGAACTTAAGC
	LR7	Reverse	TACTACCACCAAGATCT

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profileLSU

98 ^{o}C - 30 sec
98 ^{o}C - 5 sec
54 ^{o}C - 10 sec } 40 cycles

72 ^{O}C	-	15 sec
72 ^{O}C	-	60 sec
4 ^{O}C	-	∞

Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 4 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product is mixed with 0.5µl of ExoSAP-IT and incubated at 37^{O}C for 15 minutes followed by enzyme inactivation at 85^{O}C for 5 minutes.

Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol.

The Sequencing PCR mix consisted of the following components:

D/W	6.6µL
5X Sequencing Buffer	1.9µL
Forward Primer	0.3µL

Reverse Primer	0.3µL
Sequencing Mix	0.2µL
Exosap treated PCR product	1µL

SequencingPCR amplification profile

96^{O}C	-	2min	
96^{O}C	-	30sec	}
50^{O}C	-	40sec	} 30 cycles
60 ^{O}C	-	4min	
4 ^{O}C	-	∞	

Post Sequencing PCR Clean up

D/W	5 µl
3M Sodium Acetate	1 µl
EDTA	0.1 µl
100% Ethanol	44 µl

1. Mix D/W, 125mM EDTA, 3M sodium acetate pH 4.6 and ethanol were prepared and were properly mixed.
2. 50 µl of mix was added to each well in the sequencing plate containing sequencing PCR product.
3. Vortex by Mixmate vortex and Incubated at room temperature for 30 minutes
4. Spun at 3700 rpm for 30 minutes
5. Decanted the supernatant and added50 µl of 70% ethanol to each well
6. Spun at 3700 rpm for 20 minutes.
7. Decanted the supernatant and repeated 70% ethanol wash
8. Decanted the supernatant and air dried the pellet.

The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer(Applied Biosystems).

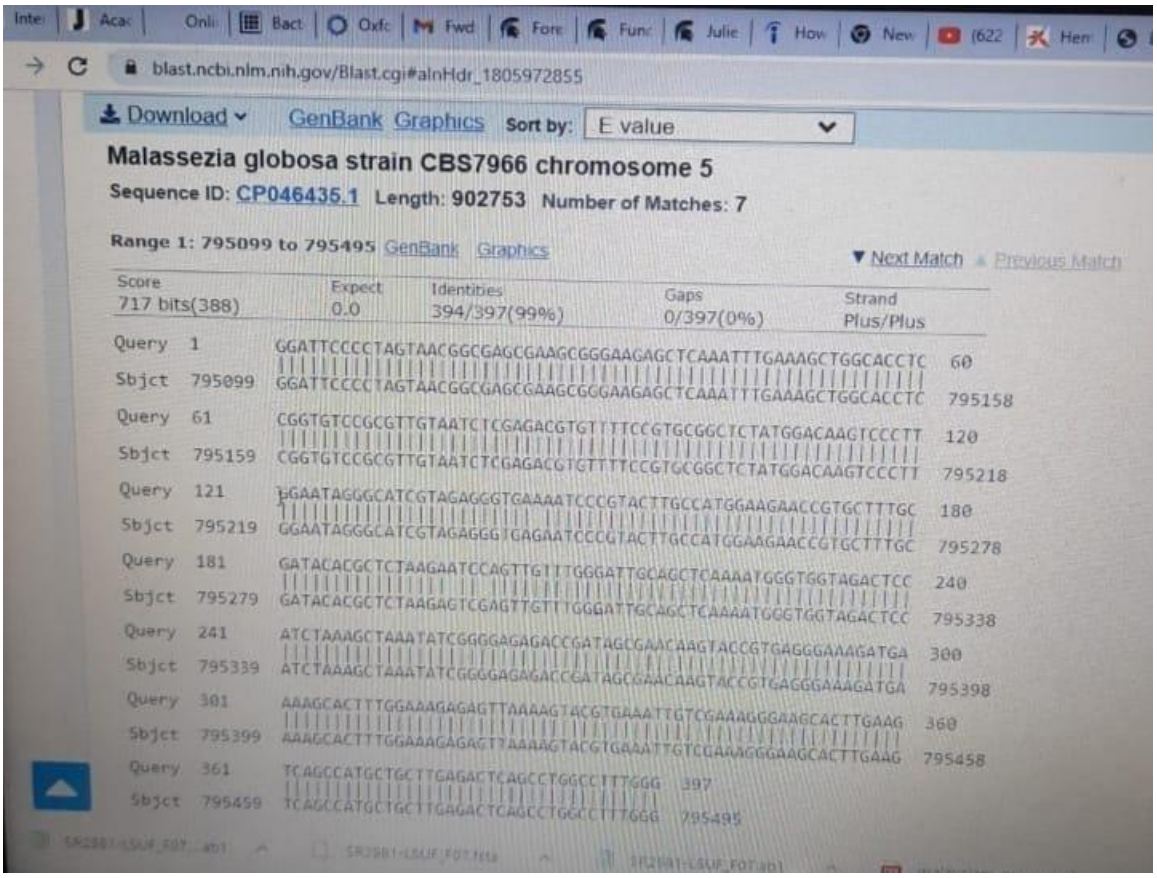
Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010).

Forward Primer

Fungi
>SR2981-LSUF_F07.ab1
GGATTCCCCTAGTAACGGCGAGCGAAGCGGGAAGAGCTCAAATTTGAAAGCTGG
CACCTCCGGTGTCCGCGTTGTAATCT
CGAGACGTGTTTTCCGTGCGGCTCTATGGACAAGTCCCTTGGAATAGGGCATCGT
AGAGGGTGAAAATCCCGTACTTGCC
ATGGAAGAACCGTGCTTTGCGATACACGCTCTAAGAATCCAGTTGTTTGGGATTG
CAGCTCAAAATGGGTGGTAGACTCC
ATCTAAAGCTAAATATCGGGGAGAGACCGATAGCGAACAAGTACCGTGAGGGA
AAGATGAAAAGCACTTTGGAAAGAGAG
TTAAAAGTACGTGAAATTGTCGAAAGGGAAGCACTTGAAGTCAGCCATGCTGCT
TGAGACTCAGCCTGGCCTTTGGGT

Nucleotide test sequence of the strain showed 99% of homology with Malassezia globosa



6. CONCLUSION

Termites are important in the ecological ecosystem as they consume and recycle woods leaf and gung. Termites which are capable of ingesting wood. The digestive system of termites consists of forgut, midgut and hindgut. The mound soil is generally more fertile than other soil. Mainly 4 bacteria are isolated from termite gut. Out of which AB4 bacteria shows antibacterial and cellulolytic activity the rest of other bacteria were eliminated and further focusing molecular identification. The bacteria [AB4] shows similarities to 10 bacillus species. From these species 1st one will selected, ie, Bacillus tropicus. In these case of termite mound the observed bacteria only shows antibacterial property cellulolytic were absent as a result the termite mound bacteria were eliminated. Termite mound and termite gut 14 species of fungus were observed in which 5 of fungus were similar in both termite mound and gut. 5 of which fungus B shows antibacterial, antifungal and cellulolytic activity. There for further focusing molecular identification was done with the fungus B and the rest of other funguses were eliminated. After molecular identification sample were identified and its name was Malassezia globossa. These fungus shows antimicrobial and cellulolytic activity were first observed from termite gut and mound. Morphological character is yellow colored powdery appearance presence of fruiting body.

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