

Isolation, Characterization and Identification of Selected Microorganisms from Wildlife Geophagic Soil: A Probe of Pros and Cons

Rilwan O. Adewale¹, Oluwatosin T. Onasanya¹, Oladele A. Oguntade², Babajide R. Odebiyi¹, Oluseun A. Akinsorotan³, Kola S. Olorunisola⁴, Shakiru O. Sule¹ and Olusesi A. Oso¹

¹Department of Forestry, Wildlife and Fisheries, Olabisi Onabanjo University, P.M.B. 0012 Ayetoro Campus, Ayetoro, Ogun State, Nigeria

²Department of Crop Production, College of Agricultural Sciences, Olabisi Onabanjo University P.M.B. 0012, Ayetoro Campus, Ayetoro, Ogun State, Nigeria

³Department of Wildlife and Ecotourism Management, Osun State University, Oshogbo, Nigeria

⁴Department of Agronomy, Faculty of Agriculture, Al-Hikmah University, Ilorin, Kwara State, Nigeria

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ABSTRACT: The search for novel antibiotics for treating human-wildlife infections has not stopped. This study isolated and characterized bacteria and fungi from wildlife geophagic termite mounds (TMs) using standard microbiological procedure. Four composite samples of TMs evident to be eaten by wildlife were randomly collected (TM₁, TM₂, TM₃ and TM₄) in addition with a composite forest soil (FS₅) that showed no sign of consumption. With the use of appropriate media, all samples were subjected to microbiological analysis covering morphological, biochemical (for bacteria), colonial and cellular morphology (for fungi). Bacteria and fungi cells isolated and characterized from respective soil samples include; *Streptomyces lydicus/Aspergillus niger* (TM₁), *Paenibacillus polymyxa/Penicillium digitatum* (TM₂), *Pseudomonas fluorescens/Fusarium spp.* (TM₃), *Bacillus subtilis/Aspergillus flavus* (TM₄) and *Acinetobacter radioresistens/Geotrichum candidum* (FS₅), all of which have both beneficial and harmful characteristics. Wildlife may be affected by pathogenic organisms when consuming TMs for medicinal reasons.

Key words: Geophagy, medicinal, microbiological, termite mounds, human-wildlife

INTRODUCTION

Microorganism are previously thought to act as the cause of human disease with no benefits, until the discovery of antimicrobials with antagonistic effect on other microbes in their surroundings, thus acting as antibiotics for treatment of diseases.¹ Antibiotics are defined as tiny organic chemical substances capable of counteracting the growth and metabolic pathway of other microbes around them.² The use of antibiotics as therapeutics though may have changed the world, however due to their abuse in the treatment of both human and wildlife infections, several available antibiotics are no longer effective in

treating diseases. This prevalent resistance of pathogens to antibiotics therefore necessitates the search for profound source(s) of antibiotics that could fight both narrow and broad spectrum population of pathogens for effective treatment of infections.

Majority of the commonly utilized antibiotics such as chloramphenicol, tetracycline, nystatin, erythromycin and streptomycin originated from nature³. Notable antimicrobials that have been isolated (from where antibiotics originate) include filamentous ascomycetes (*Aspergillus niger*), commonly employed for citric acid production,⁴ *Streptomycetes*,^{5,6} *Actinomycetes*^{6,7} and *Bacillus*.^{6,8} Some of these antimicrobials are isolated and characterized from various sources; like fish spleen,^{9,10} human skin,^{11,12} plants/vegetable,^{10,13-15}

Correspondence to: Rilwan O. Adewale
E-mail: adewale.rilwan@oouagoiwoye.edu.ng;
biotech00600@gmail.com; Phone: +2348069702417

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water,¹⁶ dumpsites,^{10,17} industrial waste¹⁸ and soil.^{19,20} In a similar way, records of the isolation of fungi from various type of soils abounds.²¹⁻²³ Advances in science and technology have also made it more possible to produce enzymes of various kind from microorganisms isolated from soil for various industrial purposes. For instance, Maravi and Kumar²⁴ described the isolation of cellulase from bacteria. Also, Ocampo *et al.*²⁵ posited the production of phytase as feed additive to enhance their animal digestion.

Since wildlife in nature receives no medications from veterinary, one could ask how wildlife self-medicates themselves, especially when they are sick in the wild. Although, there have been several hypotheses put forward to explain this phenomenon; nutrition supplementation and medication hypotheses,^{26,27} however an evaluation of wildlife geophagic soil for microbial constituents could provide in-depth insights into the mystery. Many studies on wildlife geophagy focused on elemental analysis of the consumed soil, leaving behind the aspect of microbiology. Available reports on microbiology evaluation of TMs²⁸ less detailed the possible species of microorganisms inherent in lick soils, thus creating a gap that necessitated this study.

MATERIALS AND METHODS

Study area. The study area is Old Oyo National Park (OONP) in Oyo State of Nigeria with latitude 8°10'N and 9°05'N and longitude 3°S and 4°20'E. It occupies a total land mass of 2,512 km² that formally positioned it as the fourth of the seven national parks in Nigeria and presently positioned it still as the fourth of the seventeen national parks in Nigeria after ten additional national parks were created in 2021. For easy administration, the park has five administrative ranges; Oyo Ile, Yemoso, Marguba, Tede and Sepeteri. While the head office (administrative office) is located in Oyo town, Akoto base camp serves as the main tourist camp for Marguba, Tede and Yemosho range. The park is endowed with lots of flora and fauna from where

Kobus kob was chosen as the symbol of the park, probably because of their abundance.

Sample collection. Among the termite mounds (TMs) identified to be eaten by wildlife in OONP, under the directive of tourist guides at the locations earlier reported by Adewale and Alarape,²⁹ four TMs were randomly selected for sample collection. With the aid of a sterilized hand trowel, zip lock bags and hand gloves, sub-samples comprising of different soil portions collected from top, middle and bottom layers, at the four canners of each TM were mixed together to form a composite sample from where 5g portion per TMs (TM₁, TM₂, TM₃ and TM₄) was collected. In a similar way, another sample designated as forest soil (FS₅), showing no sign of geophagy but comprising of composite mixture of sub-soil (from 10-15cm depth) from four different forest areas, within at least 2km to TM₁₋₄ was also collected. All samples collected were later stored in a cooler containing ice-flake and transported within temperature range of 4-6°C for laboratory analysis.

Sample preparation for microbial isolation. Using serial dilution techniques, supernatant solution of each of the TMs sample was prepared by first dissolving 1g of each sample in 10ml of sterile distilled water and vortexed. 1ml of the suspension/TMs was collected into another test tube and re-vortexed. All samples were serially diluted accordingly in a sterile condition by continuous transferring and vortexing of the test sample suspension from the previous test tube until 10⁻⁷ dilution was reached.³⁰

Isolation, characterization and identification of microorganisms

Bacterial isolation. 0.1 ml of test sample (at 10⁻⁷) was carefully transferred into sterile nutrient agar (NA) plates, spread with a sterile L-shaped glass rod and incubated at 37°C for 24 hours. Bacteria colonies with clear margins were selected for further purification by sub-culturing.

Bacterial characterization and identification. All isolates from each sample test were identified using Bergey's Manual of Determinative

Bacteriology.³¹ The isolates were morphologically and biochemically characterized.

Fungal isolation. Fungi cells in TMs were also isolated by serial dilution techniques using Potato Dextrose Agar (PDA), Czapek Yeast Autolysate Agar (CYA) and Yeast Extract Sucrose Agar (YES). 0.1ml of the supernatant solution of TMs was collected and poured on plates containing different agars (PDA, CYA and YES). The plates were incubated at 28°C and discrete colonies of fungi were observed for growth on various agar media after 7 days.

Fungi Characterization and Identification. Fungal Isolates (FIs) were also characterized by

colonial and microscopic morphology using the Bergey's Manual of Determinative Bacteriology.³¹

RESULT AND DISCUSSION

The result of the suspected bacteria and fungi were presented in Table 1 and Table 2. As a representative factor, one purified colony from bacteria and one purified fungal isolate from the selected four termite mounds (TM₁₋₄) and forest soil (FS₅) were characterized. The bacteria isolates (BI₁₋₅) from the respective soil samples (TM₁₋₄ and FS₅) include: *Streptomyces lydicus* (BI₁TM₁), *Paenibacillus polymyxa* (BI₂TM₂), *Pseudomonas fluorescens* (BI₃TM₃), *Bacillus subtilis* (BI₄TM₄) and

Table 1. Bacteria isolated from termite mounds (TMs) and forest soil through colony morphology and biochemical test.

Test	Parameters	TM ₁ (Isolate 1)	TM ₂ (Isolate 2)	TM ₃ (Isolate 3)	TM ₄ (Isolate 4)	FS ₅ (Isolate 5)
1. Physical Characteristics (Colony Morphology)	Form	Filamentous	Circular	Irregular	Irregular	Irregular
	Margin	Filiform	Entire	Entire	Entire	Undulate
	Elevation	Flat	Flat	Flat	Flat	Raised
	Surface	Dull	Rough	Glistening	Glistening	Glistening
	Colour	Whitish	Off white	Off white	yellowish	Cream
	Opacity	Translucent	Translucent	Translucent	Transparent	Opaque
	Texture	Dry	Butyrous	Butyrous	Butyrous	Butyrous
	Colony size (mm)	4.0	5.0	5.0	7.0	7.0
	Cell shape	Rod	Rod	Rod	Cocci	Rod
	Gram reaction	+	+	-	+	-
2. Biochemical Characteristics	Methyl red	-	-	-	-	-
	Vogesproskauer	-	+	-	+	+
	Urease	+	-	-	-	-
	Catalase	+	+	+	+	+
	Citrate	+	-	-	+	-
	Sulphide	-	-	-	-	-
	Indole	-	-	-	-	-
	Motility	-	+	+	+	-
	Glucose	NC	AG	NC	AG	NC
	Lactose	NC	AG	NC	NC	NC
	Mannitol	NC	AG	AG	AG	NC
	Galactose	NC	AG	AG	AG	NC
	Sucrose	AG	AG	NC	AG	NC
Sorbitol	NC	AG	NC	NC	NC	
Maltose	NC	AG	NC	NC	NC	
Possible Organism		<i>Streptomyces lydicus</i>	<i>Paenibacillus polymyxa</i>	<i>Pseudomonas fluorescens</i>	<i>Bacillus subtilis</i>	<i>Acinetobacter radioresistens</i>

AG (Acid and Gas produced), A (Acid produced), + (Growth), - (No growth), NC (No change)

Table 2. Fungi isolated from termite mounds (TMs) and forest soil through colonial and microscopic morphology.

Test	Parameters	Media	TM ₁	TM ₂	TM ₃	TM ₄	FS ₅
1.Colonial	Diameter	PDA	8.50	4.70	8.00	4.70	6.00
Morphology		YES	8.50	6.00	6.00	6.00	8.00
		CYA	8.50	3.30	5.00	3.30	5.00
		Obverse	PDA	Black	Green	White	Green
	Reverse	YES	Brown	Yellowish	Gray	Yellowish	Whitish
		CYA	Brown	Off white	Off white	Greenish	Gray brown
		PDA	Yellow	-	Whitish	Cream	Whitish
		YES	Yellow	Yellow	Brownish	Yellow	Cream
CYA	Cream	Yellow	Creamy	Off white	Whitish		
2.Microscopic	Head	CYA	-	-	Radiate	-	Radiate
Morphology	Stipe	CYA	Hyaline smooth	Hyaline, Smooth	Brown, Smooth	Hyaline, Smooth	Hyaline Smooth
	Vesicle	CYA	-	-	Small globose	-	Subglobose
	Phialides	CYA	+	+	-	+	+
	Conidia	CYA	+	Greenish globose	Rough brown	Greenish globose	Brown ovoid stripped
	Verticil	CYA	-	Monoverticillate	-	Monoverticillate	+
	Chlamydo-spore	CYA	-	-	-	-	+
	Arthroconidia	CYA	-	-	-	-	+
	Blastoconidia	CYA	-	-	-	-	+
Possible Organisms			<i>Aspegillus niger</i>	<i>Penicillium digitatum</i>	<i>Fusarium</i> spp.	<i>Aspegillus flavus</i>	<i>Geotricium candidum</i>

+ (Present), - (Absent), CYA (Czapek Yeast extract agar), PDA (Potato Dextrose agar) and YES (Yeast extract sucrose agar).

Acinetobacter radioresistens (BI₅FS₅). The fungal isolates (FI₁₋₅) from the soil samples (TM_{1,4} and FS₅) are: *Aspegillus niger* (FI₁TM₁), *Penicillium digitatum* (FI₂TM₂), *Fusarium* spp. (FI₃TM₃), *Aspegillus flavus* (FI₄TM₄) and *Geotricium candidum* (FI₅FS₅).

A number of microbes associated with analyzed termite mounds include some microbes that could be used to synthesize antibiotics (*Streptomyces lydicus*, *Bacillus subtilis*, *Aspegillus niger*, *Penicillium digitatum*, *Aspegillus flavus*). Other workers^{6,32,33} on TMs or soil other than TMs have reported similar microorganisms such as: *Bacillus subtilis*, *Streptomyces* and *Actinomyces* spp. as sources of antibiotics. Although the origin of some of the isolates from TMs might be traceable to the termites themselves, as Ayitso and Onyango³⁴ concluded that termite gut is the home of various filamentous bacteria of both gram positive and gram negative strains. For example, *Macrotermes bellicosus* is a

well-known fungus growing termite species used for therapeutic purpose in the Republic of Benin.³⁵ Alarape et al.³⁶ recently evaluated TMs and reported that TMs exhibited elevated antibacterial characteristics similar to that of streptomycin. Similarly, in the study carried out by Sujada et al.³⁷ termite mounds contained numerous species of *actinobacteria*, most of which belong to genus *Streptomyces*.

Isolation of microorganism associated with mineral licks were elusive until Ketch et al.²⁸ investigated colony counts of actinomycetes (non-filamentous bacteria) and fungi in TMs consumed by Chimpanzee in comparison with forest soil (control). The result revealed high proportion of non-filamentous bacteria in TMs and fungi in the control. Elevated level of clay present in the TMs than the control was suggested as possible reason for the outcome. Although, Ketch et al.²⁸ failed to identify

the microorganism to the genus level, however their observation suggested that actinomycetes constituents of the TMs could serve as stimulus for chimpanzee geophagy. In the current study, some bacteria isolates (*Streptomyces lydicus* and *Bacillus subtilis*) and fungal isolates (*Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus*), identified from the TMs are often used for the synthesis of antibiotics on industrial scale.³⁸ Similar report showed presence of *Bacillus subtilis* in soil consumed by people of Cameroon and South Africa.³⁹

CONCLUSION

Since the present day's pharmaceutical antibiotics have suffered resistance in the face of many human-animal pathogenic organisms, due to their abuse, the need to source for a new strain of highly resistant antibiotics is imperative. From the findings, it appeared that though TMs could harboured both beneficial and pathogenic microbes, but however seemed that TMs are more likely to serve as better sources of effective and highly resistant antibiotics for exploration by both the agricultural and pharmaceutical industries. Hence, the need to identify the microbes of TMs to the specie level for effective use in human-wildlife disease treatment.

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CONFLICTS OF INTEREST

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