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Research Article

Antimicrobial Activity of Bacterial Strains Isolated from *Macrotermes bellicosus* Termite Mound

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ABSTRACT

Natural environments like termite mounds can be a reservoir for novel microbial strains and antimicrobial metabolite producers. Hence, this study aimed to investigate the antimicrobial activities of bacterial strains isolated from *Macrotermes bellicosus* (*M. bellicosus*) termite mound materials. These materials were sampled from active termite mounds in the Somgandé botanic reserve in Ouagadougou, Burkina Faso. The study collected sixty-three bacterial isolates and assessed their antimicrobial activity against several pathogenic bacteria (*Bacillus subtilis, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus aureus*) and two pathogenic fungi (*Aspergillus niger* and *Candida albicans*). The dual culture and paper disc diffusion assays revealed that 10 isolates (5 bacteria and 5 actinobacteria) inhibited the growth of at least one pathogenic microorganism. In comparison, four isolates inhibited both Gram-positive and Gram-negative bacteria. Overall, isolates MBm2, MBm8 (bacteria), and MBm26 (actinobacterium) displayed better antibacterial- and antifungal activity against all tested pathogenic microorganisms. It is germane to indicate here that several typical bacteria and actinobacteria isolated from the *M. bellicosus* termite mound materials were good producers of antibacterial and antifungal agents. Thus, future studies could further characterize these isolates and optimize their growth for producing antimicrobial compounds. The bioactive compounds should also be identified for further biotechnological applications.

Keywords: Actinobacteria, Antibacterial activity, Antifungal activity, Bacteria, Macrotermes bellicosus, Termite mound material

Introduction

Despite medical progress, infectious diseases remain the leading cause of mortality [1, 2]. Since then, humanity has resorted to antimicrobial agents from plants, animals, and those of microbial origin, in the war against pathogens. In the past century, antibiotics have revolutionized modern medicine by reducing the burden of infectious diseases [2] and consequently increasing human life by 23 years [3]. However, growing reports of multidrug-resistant pathogens in the past few decades warrant serious concerns of the scientific community following the intensive overuse of antibiotics [4-6]. Therefore, we must seek out new preventive and curative antimicrobial molecules or redesign existing ones, to overcome this growing issue. For this purpose, researchers have explored diverse environments and created synthesis pathways of antimicrobial compounds to uncover novel molecules effective against these multidrug-resistant pathogens. Pharmaceutical companies have rapidly opted for chemical synthesis by a target-focused screening of synthetic compound libraries to uncover and design novel drug candidates [7]. However, the poor repertory of novel

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synthetic biomolecules has limited their strategy [7]. An alternative avenue to overcome this problem is to bio-prospect novel antimicrobial substances from microorganisms to ensure that humanity is prepared to face the increasing fatal threats of multidrug-resistant pathogens in humans, animals, and plants.

Microbial natural products are a good source of antimicrobial agents as they are more diversified (toxins, proteins, hormones, vitamins, amino acids), readily biodegradable, and less toxic to humans [8, 9]. Microbes isolated from soil, water, sewage, and sediments are potential sources of antibacterial and antifungal agents against a whole range of bacterial and fungal pathogens resistant to drugs [10]. Thus, the quest for microorganisms that produce clinically valuable, novel bioactive compounds in ecological habitats other than the ones mentioned above might prove fruitful. In fact, antimicrobial secondary metabolites have been successfully isolated from microbes living in the seabed, underground, deserts, arid soils, highlands, endophytes, plants' rhizosphere, and animals, mainly insects [11-14].

Besides the above ecosystems, researchers are also interested in termite mounds because of an abundance of actinobacteria, particularly the *Streptomyces* species. This bacterial species contributes 64-80% to the production of currently available natural antibiotics [3, 15-17]. Literature has shown that of the 22,500 microbial-derived antibiotics, approximately 45, 38, and 17% were produced by actinomycetes, fungi, and non-actinomycetes bacteria, respectively [18]. Several recent studies highlighted the robust antimicrobial activity of extracts isolated from *Macrotermes* and *Odontotermes* termite species, mounds/nests, and fungus combs [19-22]. These termites grow preferentially on a particular fungus, *Termitomyces* sp., which degrades and recycles organic matter. The latter supplies nutrients to the termites and protects them against invading entomopathogens, phytopathogens, and other fungal pathogens, thereby maintaining the well-being of the colony [20]. Um *et al.* [23] reported the isolation of a bacillaene A-producing *Bacillus* sp. from *Macrotermes natalensis* termites. This bio-substance is active against putative competitors and an antagonist of the cultivar *Termitomyces*. Additionally, *Macrotermes bellicosus* is widespread in West Africa and used in traditional Benin medicine against infectious and inflammatory diseases, as reported by Mahdi *et al.* [24]. Although the researchers did uncover interesting antibacterial activity in the compounds isolated from termites- and mound soil-derived extracts, little is known about their specific origin. This is because of the poorly documented microbial isolates from *M. bellicosus* termite mound materials, which produce these antimicrobial compounds.

In this perspective, our study aimed to isolate bacteria from the *M. bellicosus* termite mound materials and examine the isolates' antimicrobial activity. The study emphasized antimicrobial activity, which inhibits the growth of human pathogenic microorganisms, namely the pathogenic bacteria (*Bacillus subtilis, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*). This study attempts to further isolate typical bacterial strains, so far very little found, exhibiting antimicrobial activity.

Material and Methods

Sampling site and collection of termite mound material samples

Materials were collected from two active *Macrotermes bellicosus* termite mounds during the rainy season in July 2021. The sampling site is located in the botanic reserve of Somgandé (12°24'30N, 1°29'30W and altitude 294) in Ouagadougou, Burkina Faso. Sampling was carried out by excavating the mound using a 70% alcohol solution-sterilized hoe. Three samples (200 g per sample) were withdrawn using a sterile spatula at 5-10 cm depth along the internal wall of galleries (S1) and 1-5 cm from the wet surface freshly built by termites (S2). Samples were placed into sterile plastic bags, kept in an icebox, and immediately transported to the Laboratory of Microbiology and Microbial Biotechnologies, University Joseph KI-ZERBO. Samples were stored overnight at 4°C until use.

Isolation of bacteria

A composite sample of 10 g fresh termite mound materials consisting of S1 and S2 (1:1 w/w) was carried out in aseptic conditions. One gram of composite sample was suspended in 9 mL of sterile physiological saline solution (0.9% NaCl) with sterile glass beads and thoroughly vortexed for 15 min. The suspension was serially diluted 10-fold up to 10^{-8}. 0.1 mL aliquots of appropriate dilutions (10^{-3} to 10^{-8}) were spread onto

duplicate nutrient agar (NA) plates in sterile conditions. All plates were incubated at 30°C for 2-7 days.

Then, single colonies were picked up and purified both on Nutrient Broth (NB) and NA media at 30°C repeatedly until pure cultures are obtained. The pure isolates were macroscopically and microscopically checked and then kept on NA plates for short-term storage at 4°C as a working stock, and on NB medium supplemented with 20% (v/v) glycerol at -20°C for a long-term strains collection at the laboratory.

Preparation of test inoculums

The pathogenic microorganisms, namely, the bacteria *B. subtilis* ATCC 6051, *E.coli* ATCC 25922, *M. luteus* SKN 624, *P. aeruginosa* ATCC 9027, and *S. aureus* ATCC 2523, as well as the fungal strains *A. niger* ATCC 16404 and *C. albicans* ATCC 10231, were a microbial collection of the Food Technology Department (DTA) at the Research Institute in Applied Sciences and Technologies (IRSAT) in Ouagadougou. All microbial inoculums were cultured and maintained on nutrient agar (NA) and nutrient broth (NB) media, except for *A. niger* that was cultured on potato dextrose agar and broth (PDA, PDB) media. Bacterial and fungal inoculums were incubated at 30°C for 24 h and 3 days, respectively [25]. The inoculums were stored as working stock in NA media at 4°C, and in NB with 20% glycerol (v/v) for long-term storage at -20°C.

The cell density of individual colonies of each bacterium and *C. albicans* were measured by suspending each colony in 10 mL of sterile physiological saline solution before adjusting to the 0.5 McFarland turbidity standard corresponding to 10^8 CFU/mL [26, 27]. As for *A. niger*, the spores were collected from the fungal mycelia grown on a PDA medium. The mycelia were suspended in 10 mL of sterile physiological saline solution and adjusted to 10^6 spores/mL [25, 26].

Primary screening for antimicrobial activity of isolates

The antibacterial activity test was conducted according to the methods of Quintana *et al.* [27], and Salehghamari *et al.* [28] with slight modifications. Approximately, 2 cm wide of each isolate was streaked on a 9 cm Petri dish containing Mueller Hinton (MH) agar medium and incubated for 7 days at 30°C. The isolates were allowed to grow, possibly sporulate and produce the antimicrobial metabolites that diffuse through the agar. Then, a cotton swab was used to streak each of the 5 pathogenic bacteria (P1 to P5) perpendicularly to colony isolate, forming five 1 cm (width) x 5 cm (length) lines. A 1 cm space separated the isolate from pathogen agents (Figure 1A). The cultures were incubated in duplicate at 30°C for 24 to 48 h. Control plates without bacterial isolate culture were also prepared to check the normal growth of the test pathogenic bacteria on the MH medium.

This study employed a co-culture method to evaluate the antifungal test, as described by Islam *et al.* [29]. The bacterial isolates were streaked as 2 cm wide lines on the MH agar medium and incubated at 30°C for 7 days. This allowed the isolates to grow and possibly sporulate, producing the antimicrobial substances that would diffusion through the agar. Next, the pathogenic *C. albicans* (P6) was co-cultured by streaking a cotton swab of the fungus perpendicularly over the MH agar to form a 5 cm length (Figure 1B). However, the *A. niger* (P7) inoculation used a sterile paper disc (6 mm diameter) soaked in the spores/mycelia solution, before deposition on the MH agar (Figure 1C). All cultures were incubated in duplicate for 7 days at 30°C. Controls without the bacterial isolate were grown parallel to evaluate the normal growth of the fungus.

Samples which exhibited positive antibacterial and anti-yeast activity were characterized by a clear zone, denoting an inhibition zone. An inhibition zone is defined as a clear distance between test inoculums and isolates or no growth of test inoculums, compared to the controls. The percent inhibition (PI %) of radius growth of the *A. niger* for each bacterial isolate was calculated according to the formula described by Devi *et al.* [30] and Fokkema [31]:

$$PI\ \% = 100 \times (R - r)/R \quad ...(1)$$

Note:
R: Diameter of mycelial growth of *A. niger* in control plate (mm)
r : Diameter of mycelial growth of *A. niger* in test plate (mm)

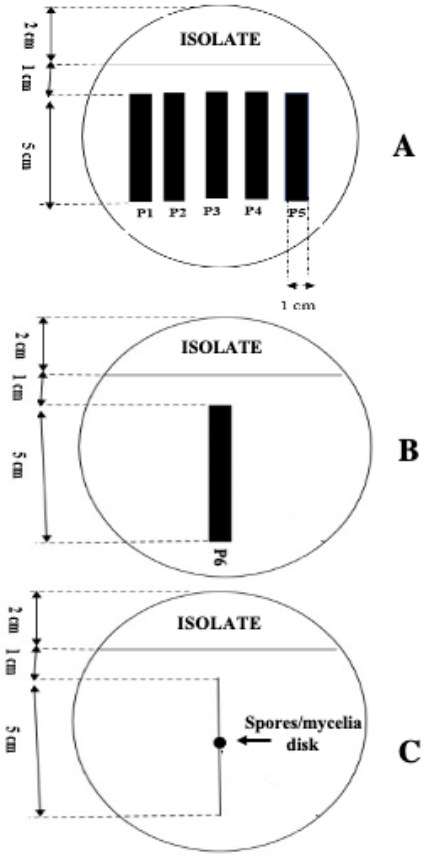

Figure 1. Streak techniques for microbial isolation and test inoculums for antibacterial (A), anti-Candida (B), and antifungal (C) activities (adapted from [27-29]). P1, P2, P3, P4, P4, P5, P6, and spores/mycelia of P7 are the test pathogens.

Fermentation culture of selected bacterial isolates

The bacterial isolates that exhibited antimicrobial activity were selected based on their ability to inhibit the growth of a broad range of the test pathogenic microbes, as seen from the large inhibition zones in the first antimicrobial test. The isolates were subsequently tested for the production of antimicrobial substances in a liquid medium, according to the methods described in the literature [32, 33], with minor modifications. Briefly, the individual colony of each isolate was inoculated in 10 mL NB and incubated for 2 days at 30°C under shaking at 150 rpm. These precultures were then inoculated in 300 mL flasks containing 90 mL fresh NB and incubated under the same conditions for 10 days. The cultures were centrifuged (7000 rpm, 15 min) [34], and their supernatants were withdrawn and filtered through a sterile Millipore syringe-filter (0.20 µm Ø). The filtrates were collected in sterile tubes and stored at 4°C for the second antimicrobial activity test.

Secondary screening for antimicrobial activity of the selected isolates

The inhibition test of the selected isolates against the pathogenic strains was conducted by a modified Kirby-Bauer disc diffusion method [23, 35]. Sterile 6 mm diameter-filter paper discs were picked up using sterile forceps and imbued with different filtrates of the isolates cultures [36]. The wetted discs were placed on the surface of MH plates immediately after seeding the standardized test inoculums. The plates were kept at 4°C for 2 h to allow the diffusion of antimicrobial substances across the MH agar, thereby temporarily inhibiting the growth of the test pathogenic strains. The pathogenic bacteria cultures were incubated at 30°C for 24 to 48 h, while the fungi were incubated for 3 to 7 days. All experiments were performed in duplicate. The positive controls for the experiment used Gentamicin (CN 120 µg/mL) and Ciprofloxacin (Cip 5 µg/mL) discs as reference antibiotics against the test bacteria, and Nystatin (NY 100 IU) discs were the reference antifungal for the test fungi. The formation of an inhibition zone confirms the inhibition of microbial activity. A transparent ruler was used to measure the diameter (mm) of the inhibition zone.

Results and Discussion

Isolation of bacteria from termite mound material samples

A total of 63 isolates were successfully isolated from the termite mound materials. The macromorphological colony characteristics revealed that most isolates were typical bacteria while few were actinobacteria (5). The typical bacteria grew rapidly in aerobic condition, after 1 to 2 days incubation on NA plates. The colonies were of notable different colors (white, brown, light brown, yellow, orange) with variable shapes (regular, irregular, round) and edges (regular, irregular, round, wavy, jagged), reliefs (flat, high) and surfaces (smooth, rough, gelatinous). Conversely, the actinobacterial isolates grew more slowly on the NA plates in aerobic condition. Their colonies were observably smaller, rough, or chalky, and formed multi-colored substrate mycelia and aerial hypha (green, white, orange, and pink-brown), similar to earlier actinobacteria reports [33, 37, 38].

Although NB and NA were used for bacterial isolation in our study, our cultivation technique allowed us to isolate both cultivable bacteria and actinobacteria. This is unlike other cultural methods that used specific media and special treatments (desiccation and calcium carbonate) to isolate only actinobacteria from various ecological niches [39, 40]. In our case, the cultivable bacteria quickly used the nutrients in the media, leading to their rapid growth in just 1-2 days. Given that actinobacteria can live and utilize available nutrients in nutrient-poor media [41], this explains their successful isolation in this study, although it was not initially expected. Therefore, their subculture onto rich media was easier [14]. However, our cultivation method yielded fewer actinobacterial isolates (5) than other previously reported literature [14, 25, 33].

Evaluation of the antibacterial and antifungal activity of isolates by the dual culture method

All antimicrobial tests were performed on MH agar media by dual culture techniques. Among the tested 63 bacterial isolates, a total of 10 isolates (MBm2, MBm5, MBm7, MBm8, MBm9, MBm10, MBm15, MBm26, MBm31 and MBm-p) (15.87%) exhibited antagonistic activity against at least one tested pathogenic strain (Table 1). A number of 6, 4, 4, 3, and 3 bacterial isolates inhibited the growth of *M. luteus*, *B. subtilis*, *P. aeruginosa*, *S. aureus* and *E. coli*, respectively. Based on cell wall type, eight (8) isolates inhibited at least one Gram-positive bacterium, while 3 isolates inhibited all the Gram-negative bacteria (Table 1). The results indicated that our isolates were more effective in inhibiting Gram-positive pathogenic bacteria than Gram-negative ones.

In fact, a similar outcome was reported by Khucharoenphaisan *et al.* [42]. These authors found that actinomycetes isolates from the termite's gut were more effective against Gram-positive than Gram-negative bacteria. Our findings also agreed with El-khawaga and Megahed [43], and Hozzein *et al.* [44]. They reported a higher inhibitory activity of desert soil microbial isolates on Gram-positive bacteria than Gram-negative ones. However, Krishanti *et al.* [14] revealed that the Pn-TN2 actinobacterial isolate from *Nasutitermes* sp. nest was more effective against Gram-negative bacteria compared to Gram-posive one's. Overall, the study found that only four isolates (MBm2, MBm8, MBm9, and MBm26) were exceptional in inhibiting both the growth of Gram-positive and Gram-negative bacteria.

Interestingly, the isolates MBm26 and MBm2 exhibited broad-spectra antibacterial activity on all the tested pathogenic bacteria, with 10 mm and 5 mm inhibition zones, respectively (Table 1, Figure 2). In contrast, the MBm9 isolate was more effective in inhibiting four pathogenic bacteria, reflected in the largest inhibition zones for *S. aureus* (20 mm), *B. subtilis* (18 mm), *P. aeruginosa* (17 mm), and *E. coli* (10 mm). The above findings corroborate earlier reports by Salehghamari and Najafi [33]. They isolated 4 isolates (act-1, act-3, act-6, and act-8) which broadly inhibited several test microorganisms such as *S. aureus* (multi-drug resistant *S. aureus*), *P. aeruginosa*, *E. coli*, *Klebsiella pneumonia*, *Serratia marcescens*, *C. albicans and A. niger*, with inhibition zones between 16 to 34 mm.

Meanwhile, the study found that isolates MBm10 and MBm15 reduced the cell density of all pathogenic test bacteria compared to controls, even though the edges of the inhibition zones were not clearly defined. This outcome was likely due to insufficient production of antibacterial compounds, which could not diffuse quickly enough into the agar. Moreover, the cell density of the pathogenic bacterial inoculums (10^8 CFU/mL) used in our experiments was higher than those used by Salehghamari and Najafi [33] (10^4 CFU/mL), and Habibeche [40] (10^7 CFU/mL).

The isolates also exhibited antifungal activity against *C. albicans* and *A. niger*, based on the respective inhibition zones of 5 to 50 mm diameter and mycelial inhibition between 25 to 100% in the dual culture assay (Table 1). Hence, all 10 isolates inhibited the growth of *A. niger*, while 5 among these isolates (MBm2, MBm5, MBm7, MBm8, and MBm15) fully inhibited the growth of fungal mycelial (100%) (Figure 3). Meanwhile, 9 isolates were effective against *C. albicans*, producing inhibition zones between 5 to 50 mm. Conversely, the MBm2 isolate only reduced the cell density of *C. albicans*, while 6 isolates (MBm8, MBm10, MBm15, MBm26, MBm31, and MBm-p) (50%) solely inhibited *C. albicans* (50 mm) (Figure 4). Isolates MBm8 and MBm15 were the most potent antifungal agents, inhibiting both *A. niger* (100%) and *C. albicans* (50 mm).

Hence, the above-said results seemed to suggest the susceptibility of our isolates to produce antifungal compounds in solid media. Prapagdee

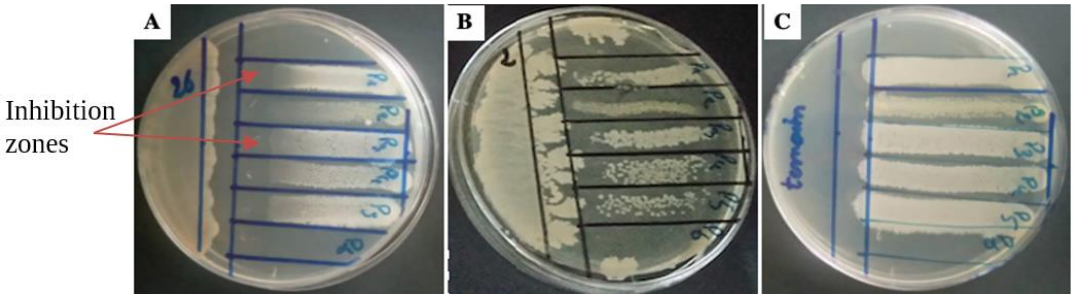

Figure 2. Antibacterial activity of isolates MBm26 (A) and MBm2 (B) against pathogenic bacteria (P1 to P5) compared with controls (C). P_1: *Staphylococcus aureus*. P_2: *Micrococcus luteus*. P_3: *Pseudomonas aeruginosa*. P_4: *Escherichia coli*. P_5: *Bacillus subtilis*.

Table 1. Antimicrobial activity of selected isolates against pathogenic inoculums by dual culture technique

Isolates	Inhibition zone (mm)						Percent inhibition (%)
	Gram-positive bacteria			Gram-negative bacteria		Yeast	Fungus
	Sa	***Ml***	***Bs***	***Pa***	***Ec***	***Ca***	***An***
MBm2	5	5	5	5	5	-	100
MBm5	0	10	0	0	0	30	100
MBm7	0	8	0	0	0	5	100
MBm8	0	0	14	13	0	50	100
MBm9	20	0	18	17	10	36	51.1
MBm10	-	-	-	-	-	50	25
MBm 15	-	-	-	-	-	50	100
MBm 26	10	10	10	10	10	50	62.3
MBm 31	0	6	0	0	0	50	35.4
MBm-p	0	8	0	0	0	50	55.5

Values are expressed as means (n=2). 0 : Absence of inhibition. More than 0 : Presence of inhibition. - : Low cell density of pathogen. *Sa* : *Staphylococcus aureus*. *Ml* : *Micrococcus luteus*. *Bs* : *Bacillus subtilis*. *Pa* : *Pseudomonas aeruginosa*. *Ec* : *Escherichia coli*. *Ca* : *Candida albicans*. *An* : *Aspergillus niger*.

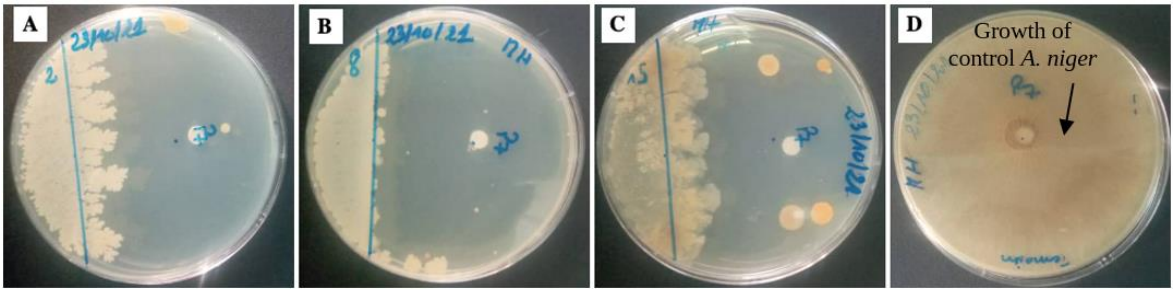

Figure 3. Inhibition potential of isolates MBm2 (A), MBm8 (B), and MBm15 (C) against *Aspergillus niger* (P7) compared with the control *A. niger* (D).

et al. [45] and Krishanti *et al.* [14] reported that bacteria/actinobacteria growing in agar media tend to release extracellular hydrolytic enzymes and other secondary antifungal compounds. These compounds then inhibited the growth of other competing fungi. The study anticipated that the microorganisms isolated from the termite mounds could synthesis antimicrobial compounds, which could suppress the antagonistic fungi of cultivar fungus *Termitomyces* [23, 30], and fungal entomopathogens (*Metarhizium anisopliae*) of termites [46], while maintaining the colony's health and survival.

Assessment of antibacterial and antifungal activity of isolates by disc diffusion method

All the 10 isolates selected from the preliminary antimicrobial test were tested for antimicrobial compounds using the paper disc diffusion method through their filtrates obtained from

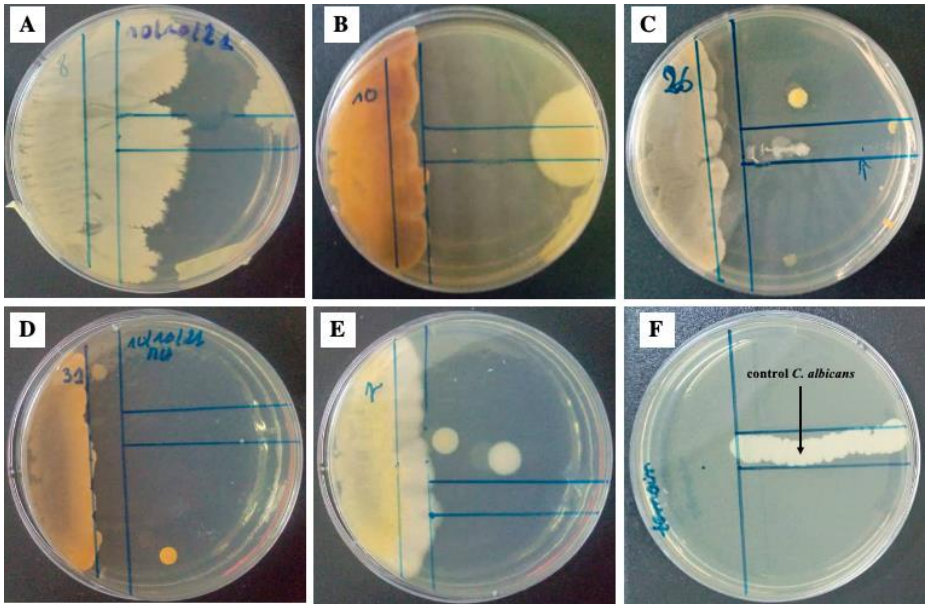

Figure 4. Inhibition potential of isolates MBm8 (A), MBm10 (B), MBm26 (C), MBm31 (D) and MBm-p (E) against *Candida albicans* compared with the control *C. albicans* (F)

fermentation cultures. For the antibacterial compounds test, 5 isolates (50%) (MBm2, MBm8, MBm26, MBm31, and MBm-p) inhibited at least one pathogenic bacterium (Table 2), with MBm8 and MBm2 showing broad-spectrum inhibition against *S. aureus* (15 and 11.5 mm), *E. coli* (12.5 and 9 mm), *P. aeruginosa* (11 and 9 mm), and *B. subtilis* (7 and 7 mm) (Figure 5). The results proved that our two isolates (MBm8 and MBm2) effectively inhibited Gram-positive and Gram-negative bacteria, except M. luteus. Duraipandiyan *et al.* [47] also reported that their soil-isolate strain ERIH-44 produced antibacterial substances against *S. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis*. In contrast, isolates MBm31, MBm26, and MBm-p only inhibited M. luteus, producing the respective highest inhibition diameters of 21, 20.5, and 12.5 mm (Figure 6).

The above-said isolates could outcompete the growth of pathogenic Gram-positive and Gram-negative bacteria in the insect nest might have arisen from one or a cocktail of antimicrobial compounds they produced. The outcome seen here corroborates studies by Ayitso *et al.* [36], Krishanti *et al.* [14], and Kroiss *et al.* [48]. However, studies that elucidate the diversity of antibacterial compounds produced by bioactive typical bacteria from termite mounds or nests are few and far between [32, 49]. By contrast, the study of Mahdi *et al.* [24] identified several antibacterial compounds from the ethanolic extracts of *Macrotermes bellicosus* soldier termites that strongly inhibited the Gram-positive *S. aureus* bacteria, as well as the Gram-negative *E. coli* and *P. aeruginosa*. Thus, further studies are needed to uncover the composition and diversity of antibacterial substances derived from termite mounds-associated bacteria.

Contrary to the dual culture results to determine antifungal activity, the disc diffusion assay revealed fewer isolates that inhibited the tested pathogenic fungi. Among the 10 potential isolates, only 2 isolates (MBm 26 and MBm15) inhibited at least one pathogenic fungi (Table 2, Figure 7), with MBm26 being the most potent isolate. The bacterium strongly inhibited the growth of *A. niger* (32.5 mm) and the pathogenic bacterium *M. luteus* (20.5 mm) while moderately inhibiting the growth of *C. albicans* (11.5 mm). Remarkably, the MBm 26 isolate inhibited all the pathogenic bacteria and fungi in the dual culture test. It is pertinent to indicate here that the study findings corroborate an earlier report by Arasu *et al.* [50], which showed the ERI 26 isolate strongly inhibiting *B. subtilis*, *P. aeruginosa*, *A. niger,* and *C. albicans* grown on solid media than in the broth media.

For the pathogenic test microorganisms, our isolates' fermentation culture filtrates might have diluted the produced antimicrobial substances when solvents were not used for their extraction. Ayitso *et al.* [36] also described a similar outcome in their *Macrotermes michaelseni* termite isolate extracts that exhibited 76.5% antimicrobial

Table 2. Antimicrobial susceptibility test of selected isolates against pathogens by the disc diffusion method

	Isolates MBm										Reference antibiotics		Reference fungicide
	2	5	7	8	9	10	15	26	31	p	CN	Cip	Nys
Pathogens	Inhibition diameter (mm)												
S. aureus (P1)	11.5	-	-	15	-	-	-	-	-	-	20.7	22.7	NA
M. luteus (P2)	-	-	-	-	-	-	-	20.5	21	12.5	26.5	34.1	NA
P. aeruginosa (P3)	9	-	-	11	-	-	-	-	-	-	25.9	26	NA
E. coli (P4)	9	-	-	12.5	-	-	-	-	-	-	25.3	28	NA
B. subtilis (P5)	7	-	-	7	-	-	-	-	-	-	26	28	NA
C. albicans (P6)	-	-	-	-	-	-	14	11.5	-	-	NA	NA	20
A. niger (P7)	-	-	-	-	-	-	-	32.5	-	-	NA	NA	NA

Values are expressed as means (n=2). The inhibition diameter includes the disc diameter (6 mm). -: No inhibition (when values = 6 mm disc). Positive inhibition is represented by values > 6 mm. CN: Gentamicin. Cip: Ciprofloxacin. Nys: Nystatin. NA: not applied.

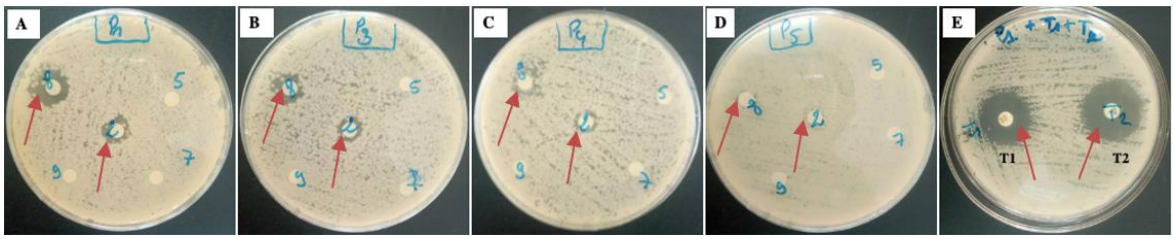

Figure 5. Inhibition zones for isolates MBm2 and MBm8 against *S. aureus* (A), *P. aeruginosa* (B), *E. coli* (C), and *B. subtilis* (D) compared to reference antibiotics (E), Gentamicin (T1), and Ciprofloxacin (T2).

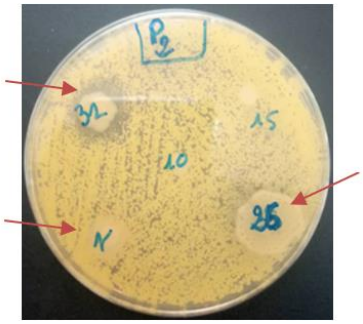

Figure 6. Inhibition zones for isolates MBm26, MBm31, and MBm-p against *M. luteus* (P2).

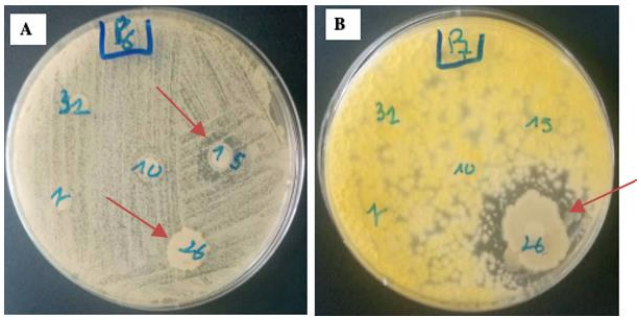

Figure 7. Inhibition zones for isolates MBm26 and MBm15 against *C. albicans* (A) and *A. niger* (B).

activity against *S. aureus* at higher fermentation culture concentrations (without dilution). Several studies observed promising antimicrobial activity in crude and organic solvent extracts of fungus-growing termites, combs, and mounds [20, 21, 23, 24, 32]. Moreover, antibiotics and antifungals production by bacteria and actinomycetes is primarily influenced by carbon and energy sources and their availability. Other factors also include the nitrogen source, cultivation conditions (pH, temperature,

NaCl concentration), and nutrients composition, as reported Iwai and Omura [51], Ruiz *et al.* [52], Sujada *et al.* [25], and Tanaka *et al.* [53].

Cell morphology observation on our 5 bacterial isolates (MBm2, MBm5, MBm7, MBm8, and MBm9) and 5 actinobacterial isolates (MBm10, MBm15, MBm26, MBm31, and MBm-p) revealed them all to be Gram-positive (unpublished data). Several studies isolated numerous antimicrobial substances-producting actinobacterial species mostly related to the *Streptomyces* genus [14, 25, 32, 54], whereas little is documented on antimicrioal agents-producing typical bacteria and the same goes for bacteria. Only Um *et al.* [23] isolated a *Bacillus* sp. bacterial strain from *Macrotermes natalensis* that secreted a single major antifungal substance, bacillaene A, which inhibited putative competition or antagonistic fungi of the cultivar *Termitomyces*. Thus, our study uncovered that the *Macrotermes bellicosus* mound materials harbor obviously enough bacteria, producing bioactive substances that inhibit pathogenic bacteria and fungi.

For a long time, most studies recognized fungal cultivars (*Termitomyces* sp.) as the leading producers of bio-substances, which role is to maintain healthy colonies by suppressing antagonistic organisms [20, 55], in addition to degrading termite digested-plant material [56, 57]. The study findings seen here communicated that our isolated typical bacteria would be also responsible for defending and preserving mound colony health. The same was observed for the actinobacteria and fungal isolates in the termite mounds, which produce antimicrobial substances as putative defensive symbionts [20, 55]. The bioactive substances produced by beneficial microorganisms inhabiting *M. bellicosus* termite mounds might have biotechnological applications in agriculture, comestible insect breeding, and medical and pharmaceutical fields. For that, futur studies are necessary to identify these interest isolates, fully perfectly apprehend the production and characterization of bioactive compounds resulting from isolates, in addition to further screenning actinobacteria from specific media.

Conclusion

This study demonstrated that 15.87% (10 out of 63) of the *Macrotermes bellicosus* mounds isolates exhibited antimicrobial activity against at least one test pathogen. We identified MBm2, MBm8, and MBm26 isolates as potential antimicrobial-producing strains that broadly inhibited pathogenic Gram-positive and Gram-negative bacteria, and fungi. Interestingly, our isolation technique allowed the isolation of both bioactive typical bacteria and actinobacteria. In conclusion, the *M. bellicosus* termite mound is a promising source of antimicrobial substance-producing bacteria and actinobacteria that might have biotechnological applications.

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