

Antifungal Activity of Isolates Obtained from the Nest Material of *Crematogaster rogenhoferi* (Mayr, 1879) (Hymenoptera: *Myrmicinae*)

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ABSTRACT

Antagonistic microorganisms were isolate from the nest material of arboreal ant *Crematogaster rogenhoferi* were used to investigate the antifungal activity against the three selected entomo pathogen fungi. About seven actionmycetes were obtained from the nest material of *Crematogaster rogenhoferi* are used to test the antifungal property against the potential growth of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* respectively. The result shows that these isolates exhibit the antifungal property against the selected fungi. During the investigation it was found that the isolates were effectively inhibit against growth of the testing fungi

INTRODUCTION

Ants as social insect live in colonies which are generally well protected by the combined defensive mechanisms of its individual members. Nest sites are generally a limited resource for ants ^[1]. *Crematogaster* ant is specific for the *Macaranga* plant ^[2,3], pine trees ^[4] and also the *Acacia* ^[5]. Nests are found in a range of sites including in soil with or without coverings, in cracks in rocks, and arboreally in trunks and twigs. The abundance of ants in tree crowns is surprising because ants, with their movable brood, were originally typical ground arthropods, specialized in building their nests in soil or using natural cavities in dead wood ^[6,7].

Ants build different types of nest structures, including above-ground mounds built from organic materials or a set of subterranean chambers. These structures may differ in their ability to insulate the colony against temperature extremes. Species forming mound nests also need organic materials and therefore are expected to display distributions that closely match those of trees and shrubs ^[8].

Nest architecture in fungus-growing ants is important not only for the ants but also for their fungal cultivars, which require particular environmental conditions ^[9,10]. In addition, the presence of nest mates is thought to increase survivorship and pathogen resistance ^[11,12] because all grooming might be facilitated by increased group size ^[13,14].

The development of strategies allowing ants to construct nest sites independently of available cavities was one of the most important evolutionary steps towards the permanent conquest of canopies ^[15]. Undetermined fungi were frequently found growing inside the nests of *Crematogaster* sp. As these fungi were only detected in well-developed in the innermost part, it seems unlikely that they stabilize the nests, as it is the case in other carton building ant species. Huxley further suggested that the ants might feed on the spores of one of the fungi, which was growing parasitically in the tubers of *Myrmecodia* spp.

The aim of the present work was to evaluate the efficacy of the isolate actinomycetes the three selected Entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii*.

MATERIALS AND METHODS

Collection Sites

Nest material of *Crematogaster rogenhoferi* were collected from the community forest of Sohmynting Village Jaintia Hills of

Meghalaya in the month of May-June (2014). The nest materials were collected in sterile container and brought to the laboratory for investigation.

Isolation

Small pieces of nest carton were taken and sterilized by dipping in 10% sodium hypochlorite for 3-5 minutes and washing in 2 or 3 series of sterile water then placed on the surface of Sabouraud Dextrose Agar (SDA) plates. These plates were incubated at room temperature (28-30 °C) and observed periodically. The growing of fungal hyphae developing from the nest disks were then transferred aseptically to a fresh SDA. The fungi were identified following sporulation and pure cultures were stored at 4 °C on SDA plate.

Morphological Identification of the Isolates

The isolated fungal strains were cultured on SDA plates at 26 °C for 7 days to observe colonial morphology. Microscopic morphological characteristics such as size and shape of conidia, mycelia septation, and pigmentation, were used to identify fungal isolates were described according to the method established by Wipornpan et al. [16].

Screening of Actinomycetes for Antifungal Activity

Testing of antifungal activity - well diffusion method

The test fungal cultures were inoculated into freshly prepared SDA plates (fungal strains) using sterile cotton swabs. The purified extract obtained by the evaporation of the ethyl acetate extract was dissolved in 1 ml, 0.2 ml phosphate buffer. Then the wells were made (about 5 mm in diameter) on the inoculated plates using cork borer and each well was loaded with 100 µl of culture supernatant. The plates containing fungal strains were incubated at room temperature for 5-7 days. After incubation, the zone of inhibition was measured and expressed as millimeter in diameter [17].

Testing of Antifungal Activity – Dual Plate Assay Method

The center of SDA plates was point inoculated with test fungi and antagonist isolates were point inoculated at the periphery of the plate. Each plate was incubated at 28 °C, for 72-96 hours in an inverted position with a slight modification. The zone of inhibition of the fungus around each isolate was measured.

RESULTS

Isolation and Morphological Identification of the Isolates

Microscopic observation (1000X magnification) after Gram's staining revealed that the isolates is a Gram-stain-positive and rod-shaped microorganism [18]. Other morphological characteristics such as colony characteristics, type of areal hyphae, aerial mass colour, and growth of vegetative hyphae, reverse side pigments, fragmentation pattern and spore formation [19] (**Figure 1**).

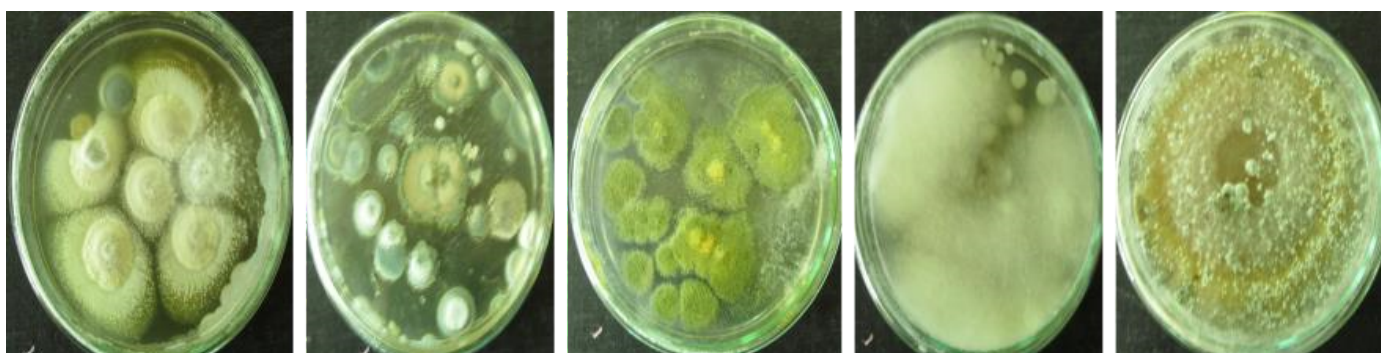


Figure 1. Morphological characteristics of isolated fungal from the nest material of *C. rogenhoferi*.

Seven fungal species were isolated from the nests material of *Crematogaster rogenhoferi*; these are *Acremonium spp.*, *Mortierella spp.*, *Mucor spp.*, *Trichoderma spp.*, *Aspergillus spp.*, *Penicillium spp.*, and *Absidia spp.* *Acremonium spp.*, *Trichoderma spp.*, *Aspergillus spp.*, *Mucor spp* and yeast *spp.* There were also certain yeasts and some non sporulating fungi that inhabited the nests material (**Figure 2**).

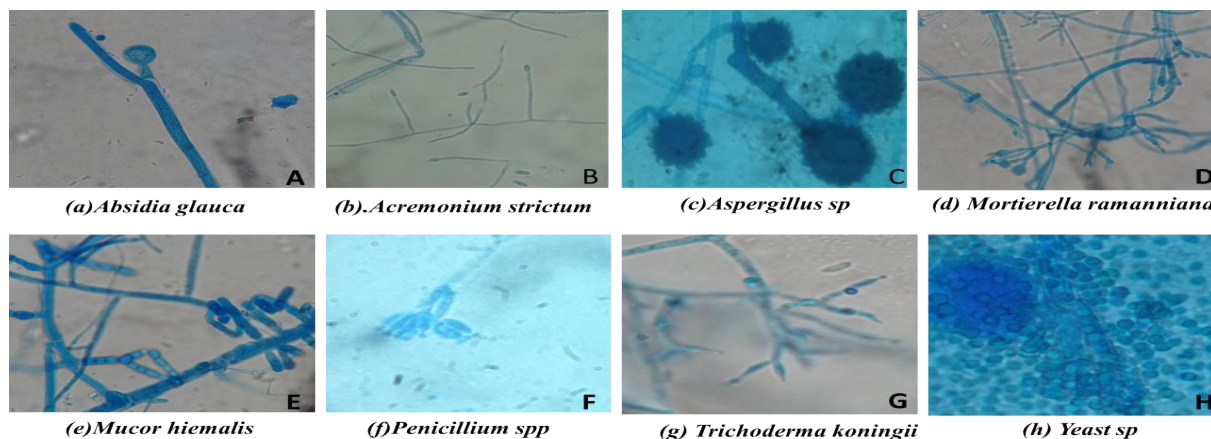


Figure 2. List of some actinomycetes isolate from the nest material shows different structures under microscopic observation (1000X magnification).

Determination of the Antifungal Activity of the Isolates

Testing of antifungal activity – well diffusion method

All seven strains of the isolates (actinomycetes) were screened against the three selected entomopathogens the *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* respectively. Amongst this 45% showed antifungal activity against the fungi. The antifungal activity of the isolates against the pathogenic fungi was good. The culture supernatant of the isolates also showed good antifungal activity (**Table 1 and Figure 3**).

Table 1. Antifungal activity of isolates actinomycetes against the three selected entomopathogens - well diffusion method.

Isolates No	Percentage of Inhibition Zone (PI) ± SD against		
	<i>Metarhizium anisopliae</i>	<i>Beauveria bassiana</i>	<i>Lecanicillium lecanii</i>
CRE101	15.4 ± 3.20	14.8 ± 1.92	15.2 ± 2.58
CRE102	13.4 ± 2.88	13.8 ± 1.30	14.2 ± 1.30
CRE103	13.8 ± 1.92	14.6 ± 2.96	14.4 ± 2.07
CRE104	16.4 ± 3.43	14.8 ± 3.76	14.4 ± 1.67
CRE105	14.2 ± 1.92	13.4 ± 2.41	13.5 ± 2.07
Control	0.4 ± 0.54	0.2 ± 0.44	0.2 ± 0.44

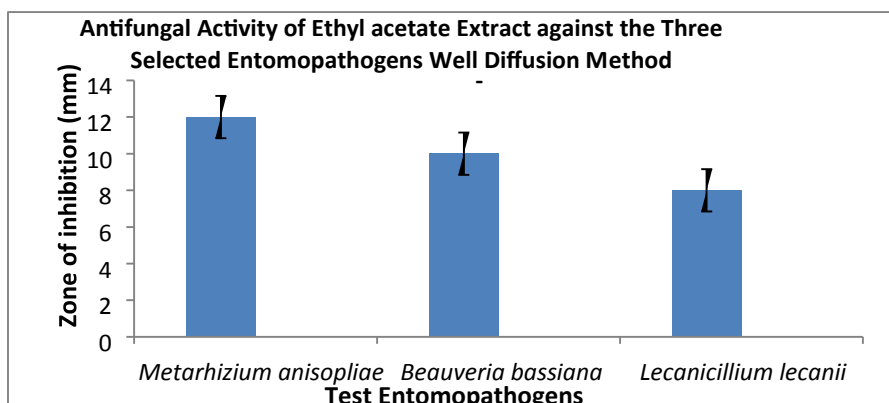
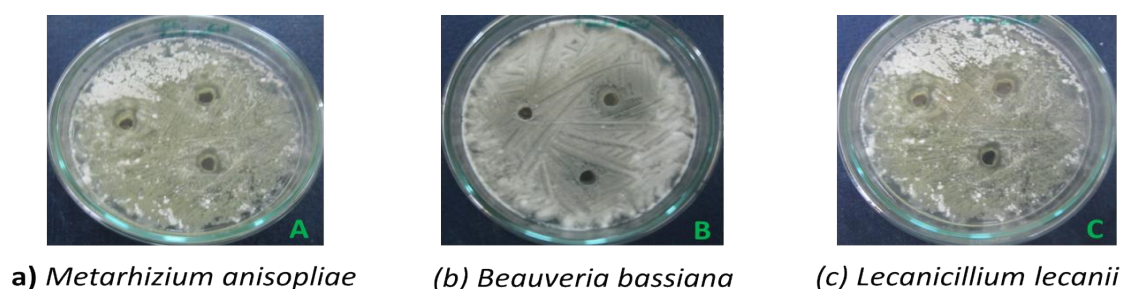


Figure 3. Antifungal activity of ethyl acetate extract against the three selected entomopathogens - well diffusion method.

(ii) Testing of antifungal activity dual plate assay method

The antifungal activity of the isolates actinomycetes was confirmed by growing with the test cultures *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* respectively. All the isolates showed antifungal activity against the test organisms was found to be effective (Table 2; Figures 4 and 5).

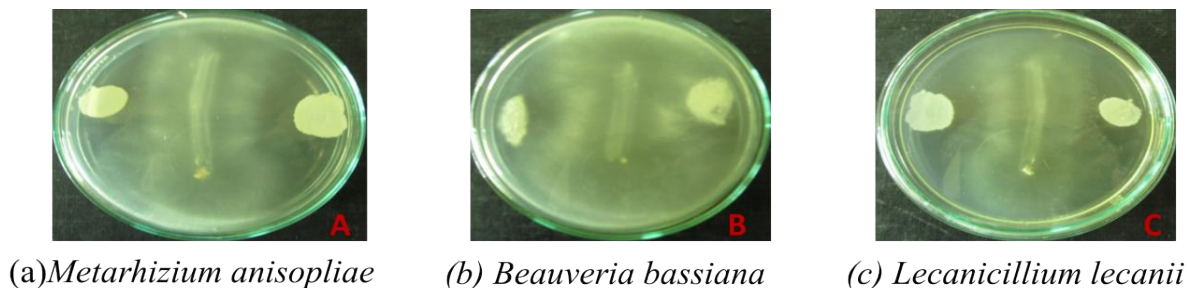


Figure 4. Antifungal activity of isolate against the three selected entomopathogens - Dual plate assay method.

Table 2. Antifungal activity of isolates actinomycetes against the three selected entomopathogens - Dual plate assay method.

Isolates No	Percentage of inhibition zone (PI) ± SD against		
	<i>Metarhizium anisopliae</i>	<i>Beauveria bassiana</i>	<i>Lecanicillium lecanii</i>
CRE101	12.33 ± 2.06	11.33 ± 2.80	10.50 ± 1.04
CRE103	9.83 ± 1.16	10.66 ± 1.63	9.83 ± 2.32
CRE105	11.50 ± 2.25	9.50 ± 1.05	10.16 ± 1.45
CRE107	11.50 ± 1.87	10.66 ± 2.65	11.16 ± 1.60
CRE109	10.67 ± 1.75	10.83 ± 1.72	12.16 ± 2.14
Control	0.51 ± 0.33	0.40 ± 0.16	0.1 ± 0.00

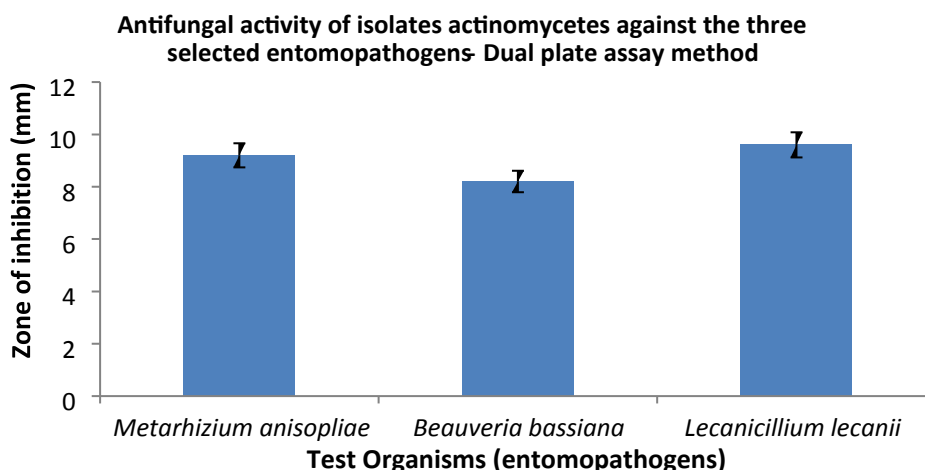


Figure 5. Antifungal activity of isolates actinomycetes against the three selected entomopathogens Dual plate assay method.

DISCUSSION

This is the first assessment to reveal that diverse actinomycetes can be readily isolated from the nest material of arboreal nest building ant. Our isolates displayed predominately gram-positive antagonism; however, it is not clear if this activity arose from one or multiple antimicrobial compounds. *Streptomyces* spp. is capable of producing over different antibiotics [20], with some strains producing multiple antimicrobials [21]. From the collected data it was well conclusive that the results indicate that the active isolates were found the produce antifungal activity against the three selected entomopathogens. This may be due to their extracellular metabolites of altering its permeability or due to the suppression of spore germination or the diffusion of antibiotic produced by local isolates into the medium which effect the growth of fungi. Because of such complexity, further studies relating to extracts from our isolates, produced under numerous culture conditions, would need to be conducted to understand the mechanism behind the bioactivity displayed in our assays.

Most of the strains we isolated belong to the genus *Streptomyces*, consistent with similar studies investigating nest-

associated insect material. This includes those studies relating to leaf-cutter ants [22-25], wood boring beetles [26-28], honey and stingless bees [29,30], solitary bees [31,32], digger wasps [33], mud dauber wasps [34-36], and termites [37,38]. Furthermore, a study by Ruddick and Williams [39], suggests that spores of *Streptomyces* spp. are associated with the cuticle of many arthropods.

While relatively little is known about how arboreal ant nest building control nest hygiene [40,41], Hoggard et al. [42] found that nest building wasps produce active cuticular antimicrobial compounds. This may preclude the need for a symbiont to produce such compounds. Our data shows to be consistent with other studies relating to actinomycetes of other insect nest substrates, suggesting arthropod nest material is a habitat for culturable, allochthonous actinomycetes that include *Streptomyces* as well as other, rarer genera [22,29,32,37,43], regardless of potential symbiotic associations. Future studies characterizing the antimicrobial activity witnessed in our studies will be necessary to determine if the actinomycetes isolated within this study produce novel chemistry. By further targeting the full diversity of the microbial community associated with these arboreal ants, we will be able to better understand how these arboreal ants maintain nest hygiene, and what microbes may impact their fitness. This growth inhibition show the active isolates of actinomycetes can be investigated for use as biofungicides. However, this strain in our study needs further investigation for active component of antibiotic production.

CONCLUSIONS

Actinomycetes isolate from arboreal ant carton nest of *Crematogaster rogenhoferi* are very potential in producing valuable secondary metabolites like antibiotics. Even though lot of antifungal antibiotics available in the market, still the problem in controlling the fungal diseases not gets an end, this is because of drug resistance by pathogens. So, in order to overcome these problems, there is a need for searching new and newer compounds from the new habitats like arboreal ant carton nest.

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