

Novel Endolichenic Fungi Isolated From *Dirinaria Applanata*

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Abstract

The conduct of this study generally aimed to isolate and characterized endolichenic fungi derived from host lichen *Dirinaria applanata*. Specifically, it sought to characterize the endolichenic fungi extracted from host lichen *Dirinaria applanata*. The host lichen was collected at Luquilu, Cabagan, Isabela. The lichen thalli were collected by detaching it from the substrata, placed inside a separate paper bag, and stored in a cool dry place (Stone et al., 2004). Identification of host lichen was accomplished through morphological characterizations including (1) presence or absence of reproductive structures (apothecia), (2) types of branches, (3) color, (4) presence of soralia, and (5) growth form. Identification through thalline spot test (K, K+C, C test) was done with the use of chemical reagents potassium hydroxide (KOH), sodium hypochlorite (C), and a combination of both (KC). The reagents were dropped directly on the exposed medulla and cortex of the lichen, and an immediate color change indicates a positive result. Endolichenic fungi were then isolated from the identified host lichen through surface sterilization. The isolated endolichenic fungi were morphologically characterized in terms of form, elevation, surface color, and underside color. The collected samples were identified and confirmed as *Dirinaria applanata*. After careful characterization, seven isolated endolichenic fungi were derived from the host lichen.

Keywords: Host lichen, *Dirinaria applanata*, endolichenic fungi, thalline spot test, surface sterilization,

Introduction

The development and discovery of drugs have been successful in controlling infections caused by bacteria. However, the increasing emergence and spread of bacterial infection pose a formidable threat to public health and welfare of people worldwide. Behera et al. (2005), stated that the challenge in the present lies on the amount of research that is necessary to find a new source of pharmacological active molecules. For this reason, the search for new and effective drugs is of necessity.

Among the alternative sources considered are lichens. Lichens are symbiotic organisms consisting of algae or cyanobacteria and fungi. Lichens are also known to harbor a variety of symbiotic fungi, known as

endolichenic fungi, which have been reported to produce bioactive secondary metabolites with pharmaceutical importance (Kosanic, 2014). Studies have shown that these metabolites also have activities against bacteria.

Among the lichens studied, *Dirinaria applanata* has been found to display marked bioactivities which have been associated with its high phenolic content and its scavenging capacity of DPPH radicals. The macrolichens of this study appear to be promising sources of bioactive compounds (Kekuda, et al., 2015)

In recent times, there have been several antimicrobial drugs on the market like Cephalosporins, Penicillin, and Pleuromutilin, which are extracted from fungi. However, the pharmaceutical industry has reduced its research efforts in infections (Norrby, Nord, & Finch, 2005) due to the limited return of investment from research and clinical trials. Also, the pharmaceutical industry has increased the price of the aforementioned drugs, making it difficult for many underprivileged Filipinos to acquire the said medication.

More specifically, no health care facility in the world is exempted from hospital-acquired infections. Individuals in low- and middle-income countries such as the Philippines experience the economic burden and such has compromised effective infection control due to lack of HAI surveillance (Maki & Zervos, 2021) Also, several studies on antimicrobial activities of endophytic fungi have been reported. However, to date, few studies on the medicinal potential of fungi have been conducted in the Philippines.

Through this research, the researcher aimed that the collected knowledge would help produce new drugs that specifically target microbial infections caused by nosocomial infections. Moreover, the gathered endolichenic fungi associated with the lichen could be utilized in creating possible novel antimicrobial drugs for Filipinos.

Given its potential as sources of novel compounds, the study generally aimed to isolate and characterize endolichenic fungi derived from host lichen *Dirinaria applanata*.

Materials and Methods

Collection and Identification of Host Lichen

The host lichen was collected at Luquilu, Cabagan, Isabela. The lichen samples collected were identified through characteristics and thalline spot tests using published identification keys and online keys and online catalogues. The lichen thalli were collected by detaching it from the substrata, placed inside a separate paper bag, and stored in a cool dry place (Stone et al., 2004).

Identification of host lichen was accomplished through morphological characterizations including (1) presence or absence of reproductive structures (apothecia), (2) types of branch, (3) color, (4) presence of soralia, and (5) growth form. Identification through thalline spot test (K, K+C, C test) was done with the use of chemical reagents potassium hydroxide (K), sodium hypochlorite (C), and a combination of both (KC). The reagents were dropped directly on the exposed medulla and cortex of the lichen, and an immediate color change indicates a positive result (Santiago et al., 2010).

Isolation of Endolichenic Fungi

The thalli from said lichens were initially rinsed with distilled water to remove excess dirt. Sterile surgical scalpel was used in order to cut the thalli into manageable portions. Surface sterilization was performed using the protocol of Li et. al (2007) with modifications to isolate the endolichenic fungi. The lichen thalli were successively treated four times with 75% ethyl alcohol for 15 seconds, and distilled water for 15 seconds, and finally with 10% NaClO for 15 seconds.

Following surface sterilization, the lichen explants were placed on Malt Extract Agar (MEA) plates (five explants per plate, in triplicates) and incubated at room temperature for 2 weeks. To check for the effectiveness of the surface sterilization method, the treated thalli explants were tissue printed on MEA. The absence of fungal growth on the tissue printed plates confirmed that the surface sterilization technique was efficient.

The fungal hypha that grew from the lichen explants were then sub-cultured using sterile syringe needles, with the aid of a dissecting microscope to ensure that single hyphae from the fungal colony was transferred onto freshly prepared MEA plates for the isolation of the endolichenic fungi. All fungal isolates were maintained at room temperature (Padhi & Tayung, 2015).

Morphological Characterization of Endolichenic Fungi

The isolated endolichenic fungi that were grown in the plates were morphologically characterized through their visible appearance under a dissecting microscope. The result of the characterization was validated by a registered microbiologist. The endolichenic fungi were characterized in terms of form, elevation, surface, margin and underside (Paguirigan, 2019).

Results and Discussion

The host lichen *Dirinaria applanata*

Lichen samples collected from Luquilu, Cabagan, Isabela were identified through characteristics and thalline spot tests using published identification keys and online keys and online catalogues (). The collected samples were identified and confirmed as *Dirinaria applanata* (Fee) A. Massal. Growth forms, type of thallus, color of the surface, and color produced by the K and C spot test were the factors considered for identification (Figure 1).

Figure 1. Host lichen *Dirinaria applanata*



Dirinaria applanata exhibits a thallus with soredia containing divaricatic acid. The dactyls were absent while orbicular soralia were present. The longitudinally rugose thallus was whitish to purple in color with epruinose or whitish pruinose apothecial disc, contiguous lobes, and flabellate apices.

According to the Consortium of North American Lichen Herbaria, *Dirinaria applanata* exhibits the following characteristics. Thallus; foliose, appressed to agglutinated, loosely appressed at the lobe tips, up to 6 cm in diameter, pinnately or subpinnately lobate lobes: radiating, confluent, flat or convex, but sometimes concave towards the lobe tips, 0.5-2 mm wide, distinctly flabellate towards the lobe tips upper surface: gray, bluish gray or almost white, with a punctiform, rarely patchy white, with a punctiform, rarely patchy white pruina or epruinose, sorediate soredia: farinose, in laminal, globose or elongated soralia pseudocyphellae: distinct, marginal, rarely also laminal, usually restricted to the peripheral parts of the lobes, sometimes reticulately confluent medulla: white, the lowest part sometimes orange, especially towards the lobe tips lower surface: black in center, paler towards lobe tips, erhizinate Apothecia: very rarely present, laminal on thallus, 0.5-1.5 mm wide disc: black, slightly grayish pruinose but mostly without a pruina ascospores: brown, 1-septate, narrowly ellipsoid, $15 \times 22 \times 6-8 \mu\text{m}$ Pycnidia: immersed in warts conidia: bacilliform, $3.5-5 \times 0.8-1 \mu\text{m}$ Spot tests: upper cortex K+ yellow, C-, KC-, P+ yellow.

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There were originally several fungal isolates that grew in the MEA media plate, however, when the specimens were subjected to characterization it was determined that some isolates exhibited similar characteristics reducing the number of isolates to seven (7). Table 1 discusses the morphological characteristics of the endolichenic fungi isolated from *Dirinaria applanata* (Figure 3).

Figure 2. Novel Isolated ELF through surface sterilization. Based on the characteristics observed, seven different strains of ELF were selected.

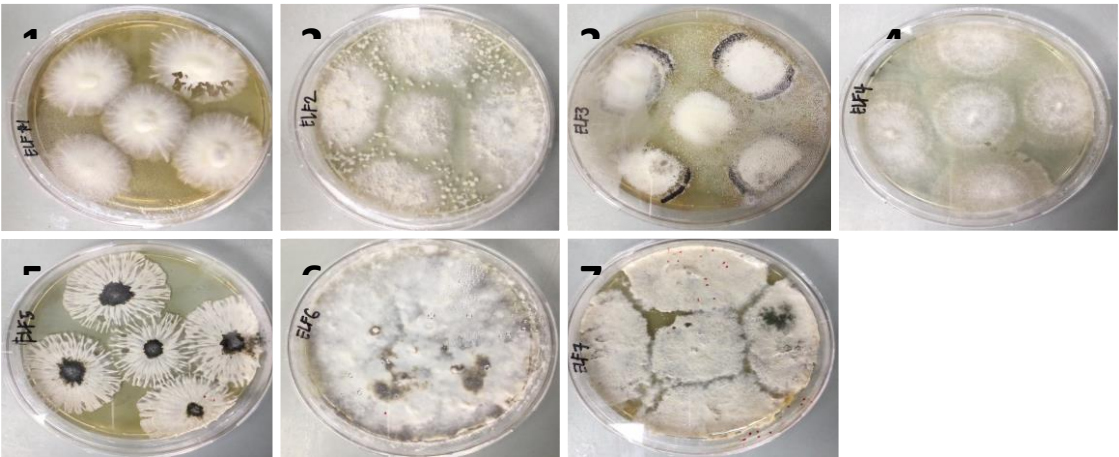


Table 1: Morphological Characteristics of Endolichenic Fungi Isolated from *Dirinaria applanata* in petri dish

Endolichenic Fungi (ELF)	Characteristics
ELF 1	Filamentous form; umbonate elevation; filiform margin; white; glistening surface; yellowish underside
ELF 2	Irregular form, flat; undulate margin, white surface; dark yellow-orange (middle)-white (margin) underside
ELF 3	Irregular form; flat; undulate margin; white with small-numerous cotton balls on surface; black (center)- yellowish (margin) underside
ELF 4	Circular form; flat; entire margin; white surface; yellowish underside
ELF 5	Filamentous form; flat; lobate margin; white surface turns black as it grows old; light-brown to black (center)- yellowish (margin) underside
ELF 6	Irregular form; raised; undulate margin; white cottony surface; yellow undersurface.
ELF 7	Irregular form; raised; undulate margin; white with small to numerous cotton balls on the surface; yellowish underside

Conclusion

There were seven novel endolichenic fungi isolated from *Dirinaria applanata*.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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