Isolation of Nematophagous Fungi from Lau Kawar Lake, North Sumatra, Indonesia

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Abstract. Root-knot nematodes are harmful organisms which cause severe damage to plants and decreasing its productivity. Biocontrol agents isolated from potential environment may solve the intriguing problem of these nematodes. Nematophagous fungi has proven as promising strategy in controlling nematodes when compared to commercial pesticides which may pollute the soil and environment through accumulated residues post-usage. Nematophagous fungi were isolated from several soils or sediments around Lau Kawar Lake using a combination of pour and sprinkle techniques. Nine fungal morphotypes were recovered from soil/sediment samples as revealed from their distinctive morphologies. Two isolates, LK.10.1 and LK.10.3 were designated as potential nematophagous fungal isolates by their effectiveness in killing nematodes within 36 hr of observation. Species-level identification is currently carried out for the isolates to reveal and confirm their identities as nematophagous fungi.

1. Introduction

Integrated pest management in horticultural field have been focusing on detrimental effects caused by root-knot nematodes [1]. These nematodes are phytoparasites living in root interiors with severe infections by decreasing absorption capacity of roots to soil nutrients and delaying growth of plants. Common agricultural pesticides, e.g. Carbofuran®, Curater™ and Petrofur™ are used to control the nematode infection in the field. However, chemical use of commercial pesticides in nematode may lead to accumulation of hazardous residues yet threatening the health status of soil [2].

Biocontrol is a technique of using antagonistic agents, through application of bacteria, fungi, virus into infected environments. Nematophagous fungi are potential agents in controlling nematodes through several mechanisms, i.e. nematode-trapping or predators, opportunistic or ovidicial endoparasites, toxin-producing fungi, and formers of special attack devices [3]. Exploration of nematophagous fungi have been conducted from many environments by mostly found in organic-rich or –poor soils in agricultural field, dung, litters and sediments [4–6].

To date, information upon nematophagous application in Indonesia is still very limited and less comprehensive. Here, we reported nine fungal morphotypes which assumed to be nematophagous fungi isolated from lake sediments in Lau Kawar, Karo regency, North Sumatra. Attempt to elaborate effectivity and potential isolates as nematophagous fungi, then the preliminary investigation was conducted to screen out several isolates through qualitative and quantitative test in this study.
2. Methodology

2.1. Field collection
Nematophagous fungi was isolated from soils or sediments nearby Lau Kawar Lake, Karo regency, North Sumatra, Indonesia (Figure 1). The lake is approximately 200 ha and a portion of 1% or 2 ha was chosen as sampling area. Soil samples or sediments were determined randomly as described from previous study [7] within each sampling points. A 0.25 m$^2$ mini-plots were placed and soils were dug up using small scoop. Triplicate plots were placed at once to collect 100 g soils prior homogenization to obtain single sample from each sampling points. Soil or sediment samples were stored in sterile plastics at 4°C prior experimentation.

![Figure 1. Map location of Lau Kawar Lake](image)

2.2. Maintenance of Caenorhabitis elegans as baits
Free-living nematodes in stock was subcultured into nematode growth medium (NGM) to obtain large individuals of *C. elegans* larvae. Culture medium was supplemented with 100 μL *Escherichia coli* OP50 as nutrition to *C. elegans*. Culture was incubated [8].

2.3. Isolation and screening of nematophagous fungi
Procedure in isolating nematophagous fungi was based on pour and sprinkle method [9,10]. One gram of soil was spreaded on top of chloramphenicol water agar (CWA) medium and incubated in the dark at ambient temperature for 3 d. After incubation, free-living nematode (*C. elegans*) in suspensions containing ± 500–1000 individuals, were poured into isolation medium and incubated for 1 d. Dead larvae observed from each samples indicate the presence of nematophagous fungi within plates as positive result. Any fungal mycelium protruding or growing on isolation medium were transferred into potato dextrose agar (PDA) medium prior further experimentation. Nematophagous fungal isolates were differentiated based on colony appearances.
2.4. Quantitative test of nematophagous fungi against *C. elegans* population

Effectivity of each nematophagous fungal isolates was tested through time-series observation and number of surviving *C. elegans* larvae. Larvae suspensions (n) approximately 50 ind/10 µL were spotted into pure colony of each fungal isolates. Number of mortal larvae was counted for every 12 hr of observation for 36 hr by immersing sterile distilled water into plate to obtain untreated-nematode suspension. Suspension was centrifuged at 1500 rpm for 5 min. Direct microscopic observation was performed on neubauer chamber under light-compound microscope with 400× magnification. This test used ten replicates divided for each interval time of observations. Results are presented in means ± standard deviations (S.Ds). Data obtained from final observation (36 hr) was analyzed using ANOVA and multiple comparisons were analyzed using Tukey test (*P* < 0.01). All statistical test and data display was performed using GraphPad Prism ver. 8.0.

3. Results and discussion

We obtain nine fungal morphotypes isolated from soil and sediments in Lau Kawar Lake using combination of pour and sprinkle method. The nematophagous started to grow in differing sequences which divided the morphotypes into fast-/slow-growing isolate under incubation for 3 d. The isolates were given codes and differentiated morphologically (Figure 1). Preliminary test using *C. elegans* as bait for nematophagous fungi showed that six of them were able to kill nematodes within 1 days of incubation. However, only two isolates namely LK.10.1 and LK.10.3 are selected as potential nematophagous fungi since they are fast-growing isolates as evidenced from its large radial colony growth on plates.

![Figure 2](image-url). Nematophagous fungal isolates isolated from sediments in Lau Kawar Lake grown on PDA medium for 3 d. (A) LK.06.1, (B) LK.06.2, (C) LK.06.3, (D) LK.10.1, (E) LK.10.2, (F) LK.10.3, (G) LK.10.4, (H) LK.10.6, (I) LK.08.2
Table 1. Qualitative screening of nematophagous fungal isolates

| Isolates | Result | Isolates | Result | Isolates | Result |
|----------|--------|----------|--------|----------|--------|
| LK.06.1  | -      | LK.10.1  | +      | LK.10.4  | +\(^a\) |
| LK.06.2  | +\(^a\) | LK.10.2  | -      | LK.10.6  | +\(^a\) |
| LK.06.3  | +\(^a\) | LK.10.3  | +      | LK.08.2  | -      |

\(^a\): slow-growing isolates

Quantitative test was subjected to two potential isolates, LK.10.1 and LK.10.3. Prolonged time of incubation was conducted to ensure the dynamic of nematicidal activity by isolates. Control was made without any fungal colony. The results showed that the two isolates almost matched the effectivity of *A. oligospora* as positive control in killing nematodes within 36 hr (Figure 3). Treatment using *Arthrobotrys oligospora* along with two isolates showed an increase of nematicidal activity during observation time which indicate their predatory activities and nutritional preferences towards *C. elegans* larvae.

![Figure 3](image)

**Figure 3.** *Caenorhabditis elegans* population (*n*) under co-incubation with nematophagous fungi (LK.10.1, LK.10.3), *Arthrobotrys oligospora* (Positive control) and None (Negative control). The bars represent standard deviation (*n* = 10).

![Figure 4](image)

**Figure 4.** Surviving *C. elegans* larvae after 36-hr incubation. The bars represent standard deviation (*n* = 10) and different letters denote significant differences at *P* < 0.01 using Tukey test.
Statistical test using ANOVA was performed to obtain any significant differences among control and treatments using nematophagous fungi. The results showed that nematicidal activities among isolates were not differed significantly which indicate the effectivity of two isolates being used as nematophagous fungi. However, no information is obtained for their nematicidal activities whether it was caused by predatory activity using trapping devices, toxin production, endoparasitic behaviour or other killing devices.

Informations of nematode-trapping fungi isolated from several region in North Sumatra has been reported by revealing a number of species namely Arthrobotrys oligospora, Candelabrella musiformis, Dactylella eudermata, and Monacrosporium eudermatum. The nematode-trapping fungi showed promising field trial results against Meloidogyne incognita as one of important root-knot nematodes in plantation and agricultural fields [11–13]. In addition, a study of freshwater nematophagous fungi also reported an assemblage of diverse fungal strains capable of killing nematodes based on the laboratory investigations [14]. Therefore, the identities of our newly-found isolates based on the molecular identification or ITS must be conducted along with its prospects through field trial test to assess their field efficacy.

4. Conclusions
Nine fungal morphotypes are evaluated and screened for its possible nematicidal activity using C. elegans larvae as baits. Two isolates, namely LK.10.1 and LK.10.3 are designated as potential nematophagous fungi as revealed from its similar effectivity to A. oligospora as positive control.

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