Occurrence of Nematophagous Fungi in Freshwater Samples of Toba Lake, North Sumatra, Indonesia

W E Tarigan, E Munir, L D S Hastuti, and A Hartanto

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia

*Email: erman@usu.ac.id

Abstract. Root-knot nematodes are important agricultural pests causing serious economical loss to harvestable crops. Biological control using nematophagous fungi is one option to mitigate these infection through mechanism of physical or chemical killing methods. The present study tried to explore a possibility of finding native nematophagous fungal strains in the hope on discovery of novel and potential isolates originating from freshwater region of Toba Lake, North Sumatra, Indonesia. Isolation of nematophagous fungi was based on sprinkle and pour combination method on Chloramphenicol-Water Agar (CWA) incubated for 30 days. Freshwater samples of soil and sediments were collected from 28 sampling sites characterized by different anthropogenic activities as natural, fishery, residential, plantation and tourism area. Daily examination is conducted to obtain a single culture of nematophagous fungi sub-cultured on Potato Dextrose Agar (PDA) medium. Eleven isolates were observed to initiate predatory activities against tested Caenorhabditis elegans based on qualitative screening. The isolates showed either mechanical killing or chemical killing of nematodes during co-inoculation with nematodes. Micromorphological and molecular analysis are currently being conducted to obtain species identity from each isolate.

1. Introduction

Biocontrol is a technique of using biological agents, such as bacteria and fungi to treat infected objectives. Nematophagous fungi are predaceous fungi with biocontrol potential in mitigating plant root-knot nematode diseases [1]. The fungi are also used in veterinarian treatment and animal husbandry to cure nematode infection in pets and livestocks [2,3]. Nematophagous fungi are potential agents in controlling nematodes through several mechanisms, i.e. nematode-trapping or predators, opportunistic or oxicidal endoparasites, toxin-producing fungi, and formers of special attack devices [4].

Nematophagous fungi may be explored from various habitats starting from agricultural and horticultural field, forest soil, organic waste while little is still known from freshwater origins [5–8]. Recent investigations revealed that freshwater habitat, e.g lake may be considered as potential source of nematophagous fungi [9,10]. This preliminary study reported a collection of nematophagous fungi isolated from freshwater region in Toba Lake, North Sumatra, Indonesia. Toba Lake being the largest and notable freshwater region in North Sumatra, may be explored thoroughly in possibility of finding new record or indigenous strains of nematophagous fungi with latter potential as biocontrol agents against Meloidogyne spp. in agricultural sector.
2. Materials and Methods

2.1. Sampling sites
Nematophagous fungi was isolated from freshwater sediments around Toba Lake, North Sumatra, Indonesia. A three $0.25 \text{ m}^2$ mini-plots were placed randomly and soils were sampled using scoops. Soil samples weighing 100 g were homogenized to obtain single sample from each sampling points [11]. Soil or sediment samples were stored in sterile plastics at $4^\circ C$ prior experimentation. Total of 28 sampling sites were grouped based on the presence (+/-) of human activities.

2.2. Maintenance of Caenorhabitis elegans as baits
Culture collection of Caenorhabitis elegans were nourished into nematode growth medium (NGM) to obtain fresh larval individuals. Culture of C. elegans was filled with 100 $\mu$L suspension of Escherichia coli OP50 served as food and medium was incubated at ambient temperature for three days [12].

2.3. Qualitative screening of nematophagous fungi
Isolation of nematophagous fungi from freshwater samples was based on previous method. One gram of soil was sprinkled on chloramphenicol water agar (CWA) medium and incubated in the dark at ambient temperature for 30 days. Bait nematodes or C. elegans larvae were poured ($\pm$ 500 ind.) into CWA medium (Figure 1). Presence of dead larvae adjacent to fungal mycelium indicate the possibility of nematophagous fungi. Fungal mycelium grew on isolation medium were then transferred into fresh potato dextrose agar (PDA) medium to improve the growth rate of isolates. Nematophagous fungal isolates were visually characterized based on morphological images of colonies.

![Figure 1. Isolation step of nematophagous using combination of pour and sprinkle method. A: Sediment sample, B: Nematode suspension](image)

3. Results and Discussions
In this study, we reported an assemblage of nematophagous fungi dominantly isolated from freshwater samples around Toba Lake, North Sumatra. Exploration around Toba Lake revealed 5 (five) habitat types based on anthropogenic activities, e.g Natural, Fishery, Residential, Plantation, and Tourist (Figure 2).
Criterion on isolating nematophagous fungi were based on the presence of predatory activities by fungal mycelium. Each fungal isolates were examined daily to observe any possible interaction between fungal hyphae and free-living nematodes. According to our isolation efforts, we obtained total of 11 nematophagous fungal isolates which are fast-growers in isolation medium revealing their adaptation to nutrient depletion and high predatory activities against C. elegans (Table 1). Mechanisms of predatory activities obtained from our isolates are revealed as mechanical trapper through formation of trapping devices and chemical killers, possibly by producing lytic enzymes or nematicidal toxins (Figure 3).

Nematophagous fungi are diverse group of predaceous fungi across species lineage. The notable nematophagous fungi are nematode-trapping fungi from Orbilliacae [13]. Variation in trapping devices of nematode-trapping fungi may differ in each species namely adhesive network, adhesive knobs, constricting rings, non-constricting rings, adhesive branches, undifferentiated/ unmodified adhesive hyphae, stephanocysts, spiny balls and acanthocytes [14,15]. Colonization of nematodes by nematophagous fungi were initially begun with immobilization using dense mycelial mats. Nematophagous fungi then will penetrate cuticle of nematodes by secreting extracellular enzymes, e.g collagenase to infect nematodes [16].
Table 1. Number of nematophagous fungal isolates from Toba Lake, North Sumatra

| No. | Code Isolate       | Origin    | Morphological images |
|-----|--------------------|-----------|----------------------|
| 1   | DT.9.2; DT.9.7; DT.26.1 | Natural  | ![Morphological images](image1) |
| 2   | DT.5.1; DT.23; DT.25.1 | Fishery  | ![Morphological images](image2) |
| 3   | DT.7.2; DT.7.3; DT.7.4 | Residential | ![Morphological images](image3) |
| 4   | DT.2.4; DT.2.3      | Tourism   | ![Morphological images](image4) |

Figure 3. Mechanisms of predatory activities by representative nematophagous fungi from Toba Lake. A: Control, B: Lytic enzymes/toxins, C: Trapping device. (Magnification at 400×)
4. Conclusions
Freshwater samples from representative sites in Toba Lake (natural, fishery, residential, tourist) are defined as potential sources of nematophagous fungal isolates. Eleven morphotypes based on colony appearances were designated as nematophagous fungi based on their predatory activities against C. elegans, as evidenced from the presence of mechanically-killed nematodes by trapping devices and lysed larvae by possible chemical or enzymatic mechanisms.

Acknowledgements
This research is fully funded by Indonesian Ministry of Research, Technology and Higher Education (KEMENRISTEK-DIKTI) through Direktorat Riset dan Pengabdian Masyarakat (DRPM) funding scheme of Penelitian Tesis Magister with contract number: 143/UN5.2.3.1/PPM/KP-DRPM/2019.

References
[1] Affokpon A, Coyne DL, Proft MD, and Coosemans J 2015 International Journal of Pest Management 61 273
[2] Maciel AS, Araujo JV, Campos AK, Lopes AE, and Freitas LG 2009 Vet Parasitol 161 239
[3] Saumell CA, Fernandez AS, Fuse LA, Rodriguez M, Sagues MF, and Iglesias LE 2015 Rev Iberoam Microl 32 252
[4] Soares FEF, Sufiante BL, and Queiroz JH 2018 Agriculture and Natural Resources 52 1
[5] Hastuti L and Faull J 2018 International Journal of Scientific & Technology Research 7 32
[6] Hastuti LDS, Faull J 2018 IOP Conf. Ser.: Earth Environ. Sci. 130 012009
[7] Hastuti LDS, Faull J 2018 Journal of Physics: Conf. Ser. 1116 050208
[8] Swe A, Jeewon R, Pointing SB, and Hyde KD 2009 Biodiversity Conservation 18 1695
[9] Hao Y, Luo J, and Zhang KQ 2004 Mycotaxon 89 235
[10] Hao Y, Mo M, Su H, and Zhang KQ 2005 Aquatic Microbial Ecology 40 175
[11] Lenaerts I et al 2008 The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences 63 242
[12] Duddington CL 1951 Transactions of the British Mycological Society 34 322
[13] Zhang KQ and Hyde KD 2014 Nematode-trapping fungi (Springer: London)
[14] Luo H, Mo MH, Huang XW, Li X, and Zhang KQ 2004 Mycologia 96 1218
[15] Luo H, Liu YJ, Fang L, Li X, Tang NH, and Zhang KQ 2007 Applied and Environmental Microbiology 72 3916
[16] Schenck S, Chase T, Rosenzweig WD, and Pramer D 1980 Applied and Environmental Microbiology 40 567