Genetic sequence analysis of *Arthrobotrys thaumasia* DS01 (*Monacrosporium thaumasium*): A new report from North Sumatra, Indonesia

L D S Hastuti*, K Berliani, M B Mulya, A Hartanto and S Pahlevi

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, North Sumatra 20155, Indonesia

*Email: liana.hastuti@usu.ac.id

Abstract. Exploration of nematode-trapping fungi (NTF) has been conducted previously in urban area, the Medan city, North Sumatra, Indonesia. The survey reported a number of NTF species inhabiting soil samples of organic wastes and decaying litter. Upon finding a suitable NTF isolate as nematode biocontrol agent, species identification is currently carried out based on molecular evidences. One morphotype named isolate DS01 is suspected as member of *Arthrobotrys* based on morphological characteristics. Molecular sequencing on rDNA or ITS-DNA region has successfully been performed. The genetic sequence was analyzed with database retrieved in NCBI, revealing the identity of *A. thaumasia/ M. thamasium*. Based on recent findings, this is the first report on indigenous strain of *A. thaumasia* from Indonesia.

1. Introduction

Root-knot nematodes (RKN) cause detrimental effects and serious problems in agricultural and horticultural field. Estimation upon 60% of zero productivity was observed from susceptible plants annually in Indonesia [1]. Disease control management of RKN still relied on the application of chemical pesticide which generally considered as hazardous chemicals. Residue of chemical pesticide may enter the aquifer zone in agricultural land leading to environmental pollution of the nature. In searching of an alternative disease prevention and management, the use of biological control agents or in specific to nematophagous fungi, may be promising as a substitute to the chemical pesticide.

Nematode-trapping fungi (NTF) is a group of nematophagous fungi, capable of mechanically and enzymatically killing RKNs [2]. These fungi may be used as biological control agent of RKN due to its diverse nature and minimum requirements in the field application. In addition, diversity and species richness of NTF has been studied dominantly in cold region while still limited to tropical region, especially in Indonesia. Nematode-trapping fungi (NTF) belonging to *Orbiliaceae*, was initially described by Nannfeldt in 1932, with the results of 288 species description within 12 genera [3]. Until now, exploration of NTF from various habitats is still progressing with expectations on finding new indigenous strains or even novel species.

During our exploration in Medan city, we have reported several NTF morphotypes collected from different soil samples, e.g decay litter and organic wastes. The fungal isolates were assumed to be NTF species based on morphological characteristics (Unpublished data). In this study, we perform a genetic sequence analysis to our isolate DS01, identified as *Arthrobotrys thaumasia* by comparing it with different strains based on rDNA sequence similarities and composition.
2. Materials and Methods

2.1. Fungal isolates
Isolate DS01 preserved in Potato Dextrose Agar (PDA) slant was re-grown prior experimentation. An agar plug of fungal mycelium was subcultured into a fresh PDA plate. Culture was incubated at ambient temperature for 7 days in dark condition or until forming a dense mycelium on agar plate.

2.2. Molecular identification of potential strain
Extraction of fungal genome is based on Wizard® Genomic DNA Purification Kit Protocol. Isolate DS01 mycelium were sampled and crushed using micropistilless in SDS Tris-HCl buffer pH 8.0 (600 µL) and Phenol : Chloroform (600 µL). Amplification of fungal genome within ITS-DNA region was using the universal primer for fungi identification, ITS-1F (5'−CTTGGTCATTAGGGAAGTAA-3') and ITS-4R (5'−TCCTCCGCTTATGATATGC-3') [4]. PCR reaction using thermal cycler was specified as follows: Pre-denaturation (95°C, 3 min), Denaturation (95°C, 45 sec), Annealing (55°C , 45 sec), Elongation (72°C, 45 sec), and Final extension (72°C, 7 min) within 35 cycles. The ITS-DNA amplicons was visualized and assessed for its quality under UV illumination on agarose gel. High quality DNA extracts were sequenced using Macrogen services (Singapore).

2.3. Bioinformatics study
The rDNA (ITS) sequence of isolate Al04SU was analyzed by comparing with other fungal ITS sequences retrieved from National Centre for Biotechnology Information (NCBI) databases. The sequences were checked using Basic Local Alignment Search Tool for nucleotide (BLASTn) [7,8]. Sequence pools were aligned using MUSCLE in software MEGA7.0 [5,6]. Genetic sequence was analyzed based on nucleotide composition and pairwise distance featured in MEGA7.0. Phylogenetic tree was constructed based on the Kimura 2-parameter statistical model by using the maximum-likelihood method with bootstrap replication 1000x [9,10].

3. Results and Discussion
Isolate DS01 is confirmed as Monacrosporium gephrophagum based on its morphological characteristics e.g conidial arrangement and shape. The isolate was recovered from soil samples around terrestrial area in Medan city, North Sumatra, Indonesia. The procedure in isolating the NTF was modified based on the combination of sprinkle and pour techniques [11,12]. The raw rDNA genome was then sequenced using Sanger dideoxy sequencing technique commercially (Table 1). The rDNA sequence of DS01 with approximate size of 615 bp, was then searched for similarities using BLAST for online databases (Table 2).

| Table 1. Raw rDNA sequence of isolate DS01 |
|------------------------------------------|
|>DS01 |
|CGTAAACAAGTTTCCGTAGGTAACCTGCGGAGATCATTACAAACAAAGTGAAGAAT |
|AAGTACCCAACCTCCTCGTGCTGGCCCTTCGGCTGCTGACTGTAACCTTTGGGTAACCA |
|AAAAACCTTTCTGGCCTGCACTGGGTCCTACCCGGCTGCACCATGCAACCCCAACCA |
|ACCTTTGGTACTAAACCATTTGCTGATAACCAAAATTTTGCAGAATGAAATCAG |
|ACCTTTGAAACGATCTCTTGTTCCGCGATCGATGAAAGAACCAGCAGAAGCAGTA |
|GTTAATGGAATTACAGAATTCACTACATGACTTTGAAACGACATTTGCGC 
|CATGGAATTCCTTTTGGGACATGCTGTGTTGAGTGCTACATTACACCCCTACGTAACCCCTGTTT |
|TGAAACCGGAGGATGTTAACACCAGCACCAGGTCTTTAAAGTTGTAAGCTCCTGCTGCTGCTC |
|TGCCCCAACCCGGAAACATGAAAAACACTACTTTGTTAAGGCGGGCGACAACCAGTACGGGCC |
|TGAACAAAACACTACCATTTTCTAAGGTTTGGACCTCAGATCAGAAAGATACCCCGCTGA |
|ACTTAAGCATATCATA |
Based on the total score on BLAST alignment results, the isolate DS01 gained the highest score or similarity with *M. thaumasium* and *A. thaumasia* with the percentage of identity reaching 99.67 and 100%, respectively. Of ten accessions displayed in BLAST, 50% are corresponding to the identity of *A. thaumasia* which may be assumed for confirmation on phylogenetic tree construction later. However, since BLAST results are needed to be qualitatively analyzed for proper identification, several accessions are removed and changed with the more suitable accession for phylogenetic tree construction. The composition of nucleotides among rDNA database and isolate DS01 is presented in Table 2. It can be seen that there is a significant number of Cytosine (C) in isolate DS01 which also happened to be in accordance with the number in EU977538.1 (*A. thaumasia*) and U51972.1 (*M. thaumasium*). The reliability of data was further confirmed by analyzing the genetic distance among accessions. The isolat DS01 was almost indistinguishable from EU977538.1 (*A. thaumasia*) and U51972.1 (*M. thaumasium*) as shown from the value of 0.000, which indicate little differences in nucleotide pairings and compositions (Table 4).

### Table 2. The ten highest similarity score based on BLAST results

| Rank | Accession | Identification       | Total Score | Query Cover | E-value | Per. Identity |
|------|-----------|----------------------|-------------|-------------|---------|---------------|
| 1    | U51972.1  | *M. thaumasium*      | 1109        | 98%         | 0.0     | 99.67%        |
| 2    | EU977538.1| *A. thaumasia*       | 1105        | 97%         | 0.0     | 100.00%       |
| 3    | U51975.1  | *M. eudermatum*      | 1096        | 98%         | 0.0     | 99.34%        |
| 4    | AB114475.1| *M. megalosporum*    | 1077        | 99%         | 0.0     | 98.38%        |
| 5    | AY773445.1| *A. sinensis*        | 1070        | 99%         | 0.0     | 98.21%        |
| 6    | AF106526.1| *A. thaumasia*       | 1068        | 98%         | 0.0     | 98.36%        |
| 7    | KT215216.1| *A. thaumasia*       | 1062        | 99%         | 0.0     | 97.89%        |
| 8    | MF948395.1| *A. microscaphoides* | 1057        | 94%         | 0.0     | 99.48%        |
| 9    | MF948394.1| *A. sinensis*        | 1048        | 93%         | 0.0     | 99.65%        |
| 10   | EU977529.1| *A. thaumasia*       | 1029        | 96%         | 0.0     | 97.99%        |

### Table 3. Nucleotide composition and rDNA database sequences used in this study

| Accession | Species                     | T(U) | C     | A     | G     | Total  |
|-----------|-----------------------------|------|-------|-------|-------|--------|
| AF106522.1| Monacrosporium elegans      | 25.3 | 23.7  | 26.6  | 24.4  | 1241.0 |
| AF106528.1| Monacrosporium eudermatum   | 25.3 | 23.8  | 25.9  | 24.9  | 1231.0 |
| AF106530.1| Monacrosporium eudermatum   | 26.2 | 23.5  | 25.7  | 24.6  | 1241.0 |
| MN717431.1| Arthrobotrys thaumasia DS01  | 24.9 | 26.0  | 27.6  | 21.5  | 615.0  |
| EU977538.1| Arthrobotrys thaumasia      | 24.8 | 26.2  | 27.2  | 21.8  | 600.0  |
| GU171370.1| Arthrobotrys scaphoides     | 25.0 | 22.9  | 26.3  | 25.9  | 1512.0 |
| KT215209.1| Arthrobotrys cladodes       | 25.4 | 22.6  | 26.2  | 25.8  | 1510.0 |
| KT215212.1| Arthrobotrys elegans       | 24.9 | 22.9  | 26.3  | 26.0  | 1518.0 |
| KT215215.1| Arthrobotrys eudermata     | 25.0 | 22.7  | 25.8  | 26.6  | 1800.0 |
| KT215216.1| Arthrobotrys eudermata     | 24.1 | 23.7  | 26.0  | 26.2  | 1522.0 |
| NR 145361.1| Arthrobotrys scaphoides    | 24.2 | 24.2  | 29.2  | 22.4  | 715.0  |
| U51945.1  | Arthrobotrys cladodes       | 23.2 | 25.5  | 26.3  | 24.9  | 995.0  |
| U51972.1  | Monacrosporium thaumasiun   | 24.8 | 26.0  | 27.5  | 21.7  | 608.0  |
| Average   |                             | 24.9 | 23.7  | 26.4  | 25.0  | 1162.2 |
Table 4. Genetic distance among inferred ingroup of *Arthrobotrys* and *Monacrosporium* species

| Accession                  | 1  | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  |
|----------------------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| AF106522.1 Monacrosporium elegans |    |     |     |     |     |     |     |     |     |     |     |     |     |
| AF106528.1 Monacrosporium eudermatum | 0.091 |     |     |     |     |     |     |     |     |     |     |     |     |
| AF106530.1 Monacrosporium eudermatum | 0.119 0.063 |     |     |     |     |     |     |     |     |     |     |     |     |
| MN717431.1 *Arthrobotrys thaumasia* DS01 | 0.084 0.056 0.087 |     |     |     |     |     |     |     |     |     |     |     |     |
| EU977538.1 *Arthrobotrys thaumasia* | 0.084 0.056 0.087 0.000 |     |     |     |     |     |     |     |     |     |     |     |
| GU171370.1 *Arthrobotrys scaphoides* | 0.105 0.069 0.100 0.069 0.069 |     |     |     |     |     |     |     |     |     |     |     |
| KT215209.1 *Arthrobotrys cladodes* | 0.102 0.059 0.090 0.065 0.065 0.023 |     |     |     |     |     |     |     |     |     |     |     |
| KT215212.1 *Arthrobotrys elegans* | 0.046 0.075 0.114 0.079 0.079 0.097 0.095 |     |     |     |     |     |     |     |     |     |     |     |
| KT215215.1 *Arthrobotrys eudermata* | 0.091 0.000 0.063 0.056 0.056 0.069 0.059 0.075 |     |     |     |     |     |     |     |     |     |     |     |
| KT215216.1 *Arthrobotrys thaumasia* | 0.082 0.065 0.101 0.011 0.011 0.074 0.071 0.084 0.065 |     |     |     |     |     |     |     |     |     |     |     |
| NR 145361.1 *Arthrobotrys scaphoides* | 0.105 0.069 0.100 0.069 0.069 0.000 0.023 0.097 0.069 0.074 |     |     |     |     |     |     |     |     |     |     |     |
| U51945.1 *Arthrobotrys cladodes* | 0.089 0.079 0.105 0.059 0.059 0.088 0.081 0.090 0.079 0.070 0.088 |     |     |     |     |     |     |     |     |     |     |     |
| U51972.1 *Monacrosporium thaumasiu* | 0.084 0.056 0.087 0.000 0.000 0.069 0.065 0.079 0.056 0.011 0.069 0.059 |     |     |     |     |     |     |     |     |     |     |     |

Based on the construction of phylogenetic trees, isolate DS01 is placed in the same position or one clade with *A. thaumasia* and *M. monacrosporium* (Figure 1). This placement confirms the identity of DS01 as *A. thaumasia* DS01 as also being supported from the previous analyses of sequence alignment and compositions. The accession number has been processed by GenBank with the accession code of MN717431.1. Based on literature review, this is the first report on the presence of *A. thaumasia* isolated from soil samples in North Sumatra, Indonesia. In comparison, the accession US1972.1. *M. thaumasiu* was a type specimen from CBS isolated from decaying root in USA [13]. Meanwhile, the closest accession, EU977538 or *A. thaumasia* was collected from decaying wood in Hong Kong during a comprehensive study of NTF diversity in terrestrial, freshwater and mangrove habitat [14].
Figure 1. Phylogenetic relationship of *Arthrobotrys thaumasia* DS01 (*Monacrosporium thaumasium*) based on rDNA region among other ingroup.

4. Conclusion
This is a first report on finding *A. thaumasia* collected from decaying litter or soil sample, originating from urban area, the Medan city, North Sumatra, Indonesia. The species identity is confirmed through genetic sequence analysis with the closest resemblance to the USA and China accessions.

Acknowledgement
The authors would like to express the highest gratitude to Indonesia’s Ministry of Higher Education, Research and Innovation, Direktorat Riset dan Pengabdian Kepada Masyarakat (DRPM) year 2018 for funding this research under scheme of Penelitian Dasar Unggulan Perguruan Tinggi with contract Number: 45/UN5.2.3.1/PPM/KP-DRPM/2018.

References
[1] Adnan A M, Suseno R, Tjitrosoma S, Hadi S, Wardojo S and Rambe A 1998 *Buletin Hama dan Penyakit Tumbuhan* 10 29
[2] Swe A, Li J, Zhang K Q, Pointing S B, Jeewon R and Hyde KD 2011 *Current Research in Environmental & Applied Mycology* 1 1
[3] Yu Z, Qiao M, Zhang Y, Qin L and Zhang K 2011 *Mycologia* 103 164
[4] Manter D K and Vivanco J M 2007 *J Microbiol Methods* 71 7
[5] Edgar R C 2004 *Nucleic Acids Res.* 32 1792
[6] Kumar S, Stecher G and Tamura K 2016 *Mol Biol Evol.* 33 1870
[7] Zhang Z, Schwartz S, Wagner L and Miller W 2000 *J Comput Biol* 7 203
[8] Morgulis A, Coulouris G, Rayselis Y, Madden T L, Agarwala R and Schaffer A A 2008 *Bioinformatics* 24 1757
[9] Felsenstein J 1985 *Evolution* 39 783
[10] Kimura M 1980 *J Mol Evol* 16 111
[11] Larsen M, Faedo M and Waller P J 1994 *Veterinary Parasitology* **53** 275
[12] Eren J and Pramer D 1965 *Soil Sci.* **99** 285
[13] Liou G Y and Tzean S S 1997 *Mycologia* **89** 876
[14] Swe A, Jeewon R, Pointing S B and Hyde K D 2009 *Biodivers Conserv* **18** 1695

