Original article KOREAN JOURNAL OF APPLIED ENTOMOLOGY

한국응용곤충학회지
Korean J. Appl. Entomol. 58(1): 9-13 (2019)
DOI: https://doi.org/10.5656/KSAE.2018.12.0.047

pISSN 1225-0171, eISSN 2287-545X

First Report of an Unrecorded Nematode-trapping Fungus, *Arthrobotrys sinensis* in Korea

Jihye Ha[1], Heonil Kang[1], Hangwon Kang[1], Donggeun Kim[2], Dongwoon Lee[3], Yongchul Kim[1,2] and Insoo Choi[1,2]*

[1]Department of Plant Bioscience, College of Natural Resource and Life Sciences, Pusan National University, Miryang 50463, Korea
[2]Nematode Research Center, Life and Industry Convergence Research Institute, Pusan National University, Miryang 50463, Korea
[3]School of Ecology and Environmental System, College of Ecology and Environmental Science, Kyungpook National University, Sangju 37224, Korea

국내 미기록 선충포식성 곰팡이 *Arthrobotrys sinensis*의 형태 및 분류

하지혜[1] · 강헌일[1] · 강항원[1] · 김동근[2] · 이동운[3] · 김용철[1,2] · 최인수[1,2]*

[1]부산대학교 생명자원과학대학 식물생명과학과, [2]부산대학교 생명산업융합연구원 선충연구센터, [3]경북대학교 생태환경대학 생태환경관광학부

ABSTRACT: Nematode-trapping fungi use various specialized traps to capture nematodes. A fungus that can capture nematodes in three dimensional adhesive networks was isolated from the soil around the root of *Cucumis melo* L. (Oriental melon) in Seongju, Korea. The conidiophores were found to be septate, hyaline, erect and 290-528 (342.8) µm high. It produces obovoid shape and 1-3 septate (commonly 2-septate) conidia with a size of 30.5 × 20.3 µm. Molecular analysis of 5.8 S rDNA displayed 99% similarity to *Arthrobotrys sinensis*. On the basis of morphological, morphometric and molecular studies, the fungus was identified as *A. sinensis*. It is the first report in Korea which can be one of biological control resource of plant-parasitic nematode.

Key words: Adhesive network, *Arthrobotrys sinensis*, Biological control, Nematode-trapping fungus

초 록: 선충포식성 곰팡이는 선충을 포획하기 위하여 다양하고 특수한 기관을 사용한다. 국내 성주지역의 참외 경작지 뿌리 주변의 토양에서 3차원 접착 고리를 형성하여 선충을 포획하는 곰팡이를 분리하였다. 곰팡이의 미세형태 구조를 관찰한 결과 분생포자병은 직립형으로 길이는 290~528 (342.8) µm으로 길었으며, 계란형의 30.5 × 20.3 µm 크기를 가진 1~3개의 분생포자를 형성하였다. 균주의 rDNA의 5.8 S 영역의 염기서열을 분석한 결과, *Arthrobotrys*속의 계통군에 속하였으며, 특히 *Arthrobotrys sinensis*와 99%의 유사성을 보였다. 형태적 특징과 분자생물학적 계통 분석을 바탕으로 본 균주는 *A. sinensis*로 확인되었으며, 이는 국내 미기록종으로 식물기생성선충의 생물학적 조절을 위한 하나의 자원이 될 수 있다.

검색어: 끈끈이그물형, *Arthrobotrys sinensis*, 생물학적 방제, 선충포식성 곰팡이

Nematophagous fungi have worldwide distribution, in all habitats and climates (Gray, 1987). They capture nematodes with sophisticated trapping structures, such as adhesive nets and adhesive columns, stalked adhesive knobs, constricting rings, non-constricting rings, and sessile adhesive knobs (Liu et al., 2014). Due to its specialized trapping technique, this fungus can be stand as potential biological control agents against plant parasitic nematodes (Ahrén and Tunlid, 2003). During the nematode survey, we isolated a nematode trapping fungus from the soil around the root of Oriental melon (*Cucumis melo* L.) in South Korea. And, here we describe characteristics of Korean isolate.

Materials and Methods

Nematode culture

Fresh soil around the root of *C. melo* was collected from

***Corresponding author:** Ichoi@pusan.ac.kr
Received October 7 2018; **Revised** December 4 2018
Accepted January 7 2019

greenhouse in Seongju-gun, Gyeongsangbuk-do, South Korea. The nematode-trapping fungi were separated from soil by the modified sprinkling-baiting technique (Barron, 1977). Subsamples of 1.0 g soil were sprinkled onto plates of both 1.7% corn meal agar (CMA, Difco) and 1.5% water agar (WA). About 100 nematodes (*Rhabditis* spp.) were added to the surface of CMA and WA media plates as a bait to enhance isolation of nematode-trapping fungi. CMA plate and WA plate of three respectively were used for each soil sample. The plates were incubated at 25℃ for 14 days and observed every other day under a stereo microscope (SZX 16, Olympus, Japan) to detect the appearance of nematophagous fungi. All detected nematode-trapping fungi were photographed using a compound microscope (BX53, Olympus, Japan) equipped with microscope digital camera (DP73, Olympus, Japan) and transferred to CMA for pure culture.

DNA extraction

From pure culture, genomic DNA of fungi was extracted based on methodology described by Zhu et al. (1993). The DNA of the nematode-trapping fungus was characterized by sequencing ITS region included the 5.8 S rDNA. Primers for ITS region amplification were ITS1 (5'- TCCGTAGGTGAAC CTGCGG-3') and ITS4 (5'-CCTCCGCTTATTGATATGC-3') (White et al., 1990). Polymerase chain reaction (PCR) amplification was performed in a 50 µl final volume containing 1 µl of DNA template, 1 µl of 10 pM of each primer, 4 µl of 2.5 mM dNTPs, 0.3 µl of Taq DNA polymerase, and 5 µl of 10x PCR buffer in 37.7 µl of sterile deionized water. The genomic DNA was used as a template for PCR as follows: after an initial 3 min denaturation step at 94℃, a 35 cycle amplification (94℃ for 1 min, 54℃ for 1 min, and 1 min at 72℃) was conducted. The final extension was continued for 8 min at 72℃. PCR products of amplification of DNA by PCR were confirm by electrophoresis and purified with the DokdoPrepTM Gel Extraction Kit (ELPIS Biotech, Korea). PCR amplicons were followed by nucleotide sequencing (Cosmogentech, Korea).

In order to confirm the species, the sequence of nematode-trapping fungus was compared by the BLAST sequence alignment software of the NCBI database (National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Results of the closest nucleotide sequences were selected to BLASTn analysis, the combined sequences of ITS region exhibited 99% similarity to *A. sinensis* strain 105-1 (AY773445.1). To differentiate previous isolates, we designated query strain as strain SJCM-1. Phylogenetic analyses were performed by MEGA version 6.10 with the neighbor-joining (NJ) statistical method (Saitou and Nei, 1987) and the Jukes-Cantor model (Campos et al., 2010; Falbo et al., 2013).

Results and Discussion

Taxonomic changes of species are as follows:

Arthrobotrys sinensis (Xing Z. Liu & K.Q. Zhang, 1999) M. Scholler, Hagedorn and A. Rubner, Sydowia 51: 104 (1999) = *Monacrosporium sinense* Xing Z. Liu & K.Q. Zhang, Mycol. Res. 98: 863 (1994)

The species was originally described by Xing Z. Liu and K.Q. Zhang (1999) from field soil in Huaxi, China (Yu et al., 2014). This fungus grows rapidly and form white colony. The diameter of hyphae was 4.2-9.8 (6.8) µm. The conidiophore was measured as 290.4-528.6 (342.9) µm in length, 4.2-5.5 (4.8) µm wide at the base, tapering to 2.5-2.8 (2.6) µm wide at the apex. There were few branched conidiophores. The fungus produces obovate shaped 1-3 septate conidia on CMA, 30.5 µm (27.3 - 32.8) long, and 20.3 µm (17.6 - 22.4) µm wide (Fig. 1C-G). This fungus forms a predaceous organ of three dimensional adhesive networks (Fig. 1H). The adhesive networks are produced on CMA medium either in the presence of nematodes or without nematodes (Fig. 2A and B).

The 590 bp sequence that was obtained was aligned for comparison with other sequences. A BLASTn search of the SJCM-1 strain on the ITS region revealed high-scoring matches with some *Arthrobotrys* species, the most similar to *A. sinensis* (GenBank accession number AY773445) which was 99% identical to SJCM-1 strain. The tree was constructed based on the ITS region with 5.8 S rDNA sequences with the NJ algorithm by applying the Jukes-Cantor model implemented in MEGA version 6.10 with 1000 bootstrap replications. The phylogenetic relationships of the SJCM-1 strain which are more similar to *A. sinensis* are shown in Fig. 3.

The nematophagous fungi have been described approximately 700 species (Yu et al., 2014). In Korea, 8 species of *Arthrobotrys*

(*Arthrobotrys amerospora*, *A. arthrobotryoides*, *A. brochopaga*, *A. conoides*, *A. dactyloides*, *A. musiformis*, *A. oligospora*, *A. vermicola)* have been reported (Wu and Kim, 2010). The *A. sinensis* is a new species to Korea. This fungus was almost consistent with the previous original fungus described by Liu and Zhang (Table 1), however there were some differences such as the conidiophores were longer and the size of the conidia was large etc. This fungus closely resembles to *A.*

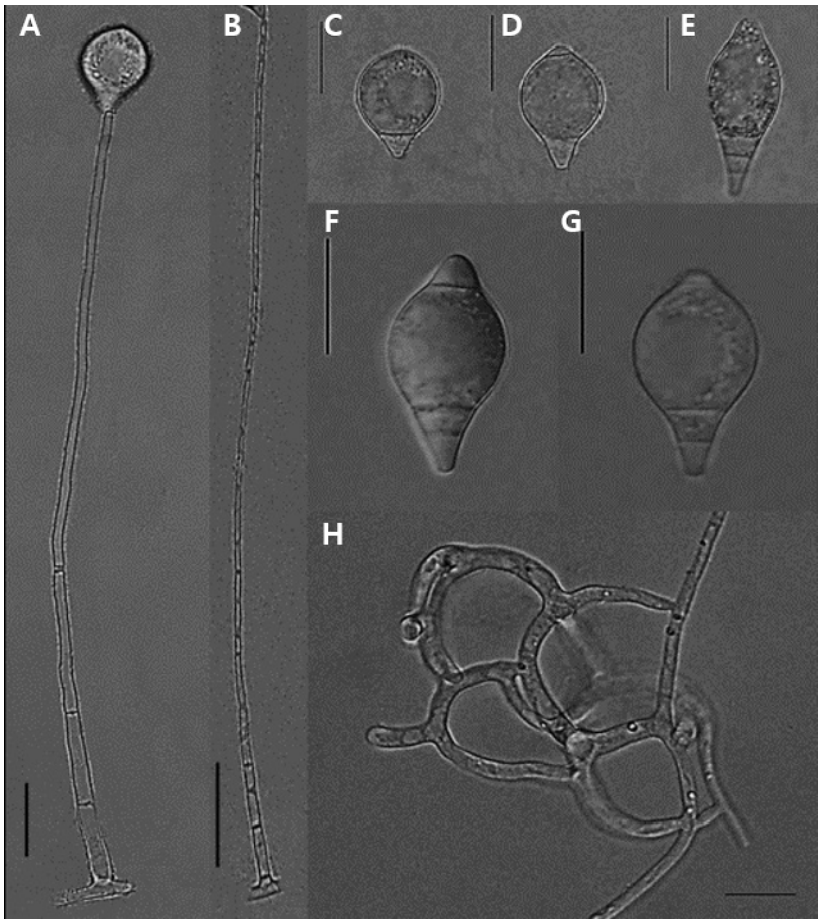

Fig. 1. *Arthrobotrys sinensis*. A-B, conidiophore; C-G, conidia; H, adhesive network. Bars = 20 μm (A, C, D, E, F, G, H) and 50 μm (B).

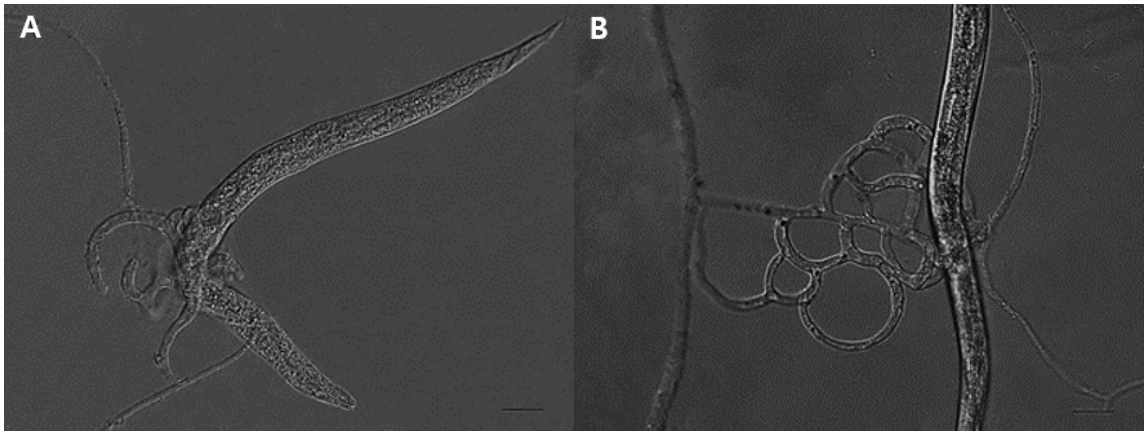

Fig. 2. Nematode captured by trap (adhesive network). Bars = 20 μm.

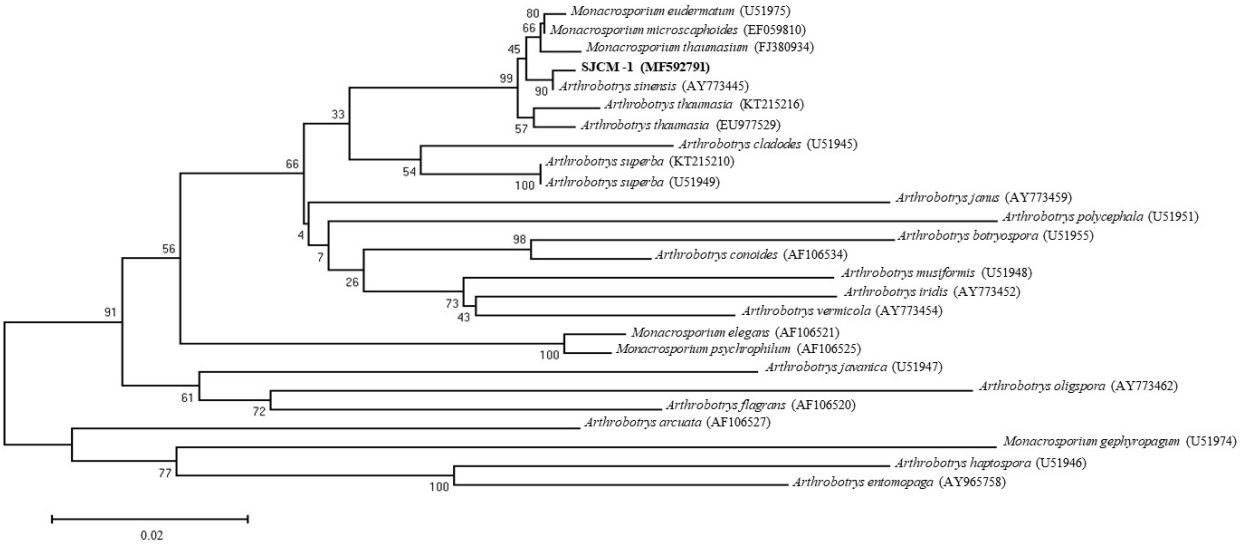

Fig. 3. Phylogenetic tree based on ITS sequence showing the position of strain SJCM-1 (MF592791) and related fungal taxa. Numbers at branches are bootstrap values, derived only for the nodes supported by greater than 50% (1,000 replicates). Bar, 0.02 substitutions per site.

Table 1. Morphological characteristics of different isolates *Arthobotrys sinensis* and its comparison to Korean isolate

Organs			Korea isolate	Original description (Xing Z. Liu and K.Q. Zhang, 1999)	*A. cookedickinson* (Cooke and Dickinson, 1965)	*A. indica* (Chowdhry and Bahl, 1999)
Conidi-ophores	Length (μm)		290-528 (342.8)	200-500	60-420	150-350
	Diameter (μm)	Base	4.2-5.5 (4.8)	4.5-5.5	5-7.5	3-6
		Tip	2.5-2.8 (2.61)	2.5-3	3-5	1-2
Conidia	Length (μm)		27.3-32.8 (30.5)	23.5-30 (27.6)	30-52.5 (42)	17.5-30 (23.2)
	Width (μm)		17.6-22.4 (20.3)	17-25 (20)	15-22.5 (17.6)	12.5-20 (14.8)
	Septa		1-3, mainly 2	1-3, mainly 2	1-3, mainly 2-3	0-2
	Shape		Subglobose, obovate	Subglobose, obovate	Turbinate, subglobose, obovate, fusiform	Ellipsoid, broadly turbinate
Form of predaceous organ			Three dimensional adhesive networks	Three dimensional adhesive networks	Three dimensional adhesive networks	Three dimensional adhesive networks

cookedickinson and *A. indica* in its relatively small conidia and conidial shape, but differs in size of conidia (27.3-32.8 × 17.6-22.4 μm) (Yu et al., 2014). The *A. sinensis* shows a rapid growth rate. When the nematode is added to the culture medium of these strains, a trap of three-dimensional adhesive nets is formed within one to two days. Here in study, we report the unrecorded nematode-trapping fungus species *A. sinensis* for the first time in Korea, and it can be a used as one of the biological control resources and a potential option for the management of the plant-parasitic nematodes.

Acknowledgements

This work was supported by a 2-Year Research Grant of Pusan National University.

Literature Cited

Ahrén, D., Tunlid, A., 2003. Evolution of parasitism in nematode-trapping fungi. J. Nematol. 35(2), 194-197.

Barron, G.L., 1977. Nematode-destroying fungi, in: Barron, G.L. (Ed.), Topics in Mycobiology No.1. Lancaster Press, Inc., Lancaster Pennsylvania, pp. 140.

Campos, R.A., Boldo, J.T., Pimentel, I.C., Dalfovo, V., Araújo, W.L., Azevedo, J.L., Vainstein, M.H., Barros, N.M., 2010. Endophytic and entomopathogenic strains of *Beauveria* sp. to control the bovine tick *Rhipicephalus* (Boophilus) *microplus*. Gen. Mol. Res. 9(3), 1421-1430.

Falbo, M.K., Soccol, V.T., Sandini, I.E., Vicente, V.A., Robl, D., Soccol, C.R., 2013. Isolation and characterization of the nematophagous fungus *Arthrobotrys conoides*. Parasitol. Res. 112(1), 177-185.

Gray, N., 1987. Nematophagous fungi with particular reference to their ecology. Biol. Rev. 62, 245-304.

Liu, K., Zhang, W., Lai, Y., Xiang, M., Wang, X., Zhang, X., Liu, X., 2014. *Drechslerella stenobrocha* genome illustrates the mechanism of constricting rings and the origin of nematode predation in fungi. BMC Genomics. 15, 114.

Liu, X.Z., Zhang, K.Q., 1994. Nematode-trapping species of Monacrosporium with special reference to two new species. Mycol. Res. 98(8), 862-868.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4(4), 406-425.

Scholler, M., Hagedorn, G., Rubner, A., 1999. A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. Sydowia 51(1), 89-113.

White, T.J., Bruns, T., Lee, S. Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a guide to methods and applications. Academic Press, San Diego, pp. 315-322.

Wu, H.Y., Kim, D.G., 2010. First report of an unrecorded nematode-trapping fungus species *Monacrosporium phymatopagum* in Korea. Plant Pathol. J. 26(3), 264-266.

Yu, Z., Mo, M., Zhang, Y., Zhang, K.Q., 2014. Taxonomy of nematode-trapping fungi from Orbiliaceae, Ascomycota, in: Zhang, K.Q., Hyde, K.D. (Eds.), Nematode-Trapping Fungi. Fungal Diversity Research Series 23. Springer, Netherlands, pp. 41-210.

Zhu, H., Qu, F., Zhu, L.H., 1993. Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. Nucleic Acids Res. 21(22), 5279-5280.