한 국 균 학 회 지 The Korean Journal of Mycology Research Article

Marine *Athrinium* spp. Isolated from *Sargassum* sp. (Brown Algae) in Jeju Island and Unrecorded Species in Korea

Seokyoon Jang, Joo-Hyun Hong, Young Mok Heo and Jae-Jin Kim*

Division of Environmental Science and Ecological Engineering, College of Life Science and Biotechnology, Korea University, Seoul 02841, Korea

ABSTRACT : *Arthrinium* (*Ascomycota*, *Apiosporaceae*) is a major marine fungal genus. Three *Arthrinium* species were reported previously, but not fully described. We isolated the two species *A. arundinis* and *A. saccharicola* from *Sargassum* sp. brown algae in Jeju Island, Korea. These species have not been previously reported in Korea. We described these species in detail and performed a phylogenetic analysis based on the nucleotide sequences of the EF1-α and β-tubulin genes.

KEYWORDS : *Arthrinium*, Marine fungi, Taxonomy, Translation elongation factor 1-α, β-tubulin

Introduction

Arthrinium Kunze (=*Apiospora* Sacc.) is a fungal genus which contains about 40 species (Index Fungorum: http://www.indexfungorum.org). They are known as plant-associated fungi, but also easily isolated from various substrates such as soil, bamboo, air and marine habitats [1-4]. Marine environment and seaweed are supposed major habitat of *Arthrinium* because four *Arthrinium* species were isolated from same seaweed at the same time [4].

Remarkable biological activities of some *Arthrinium* strains had been reported. *A. saccharicola* KUC21221 showed strong cellulolytic enzyme, antifungal activity and antioxidant capacity [4]. Other *Arthrinium* spp. showed high antifungal activities as well.

In Korea, three *Artrinium* species, *A. arundinis* (Corda) Dyko & B. Sutton, *A. phaeospermum* (Corda) M.B. Ellis and *A. saccharicola* F. Stevens, were previously reported, but have been not fully described [1, 4, 5]. Thus, we have explored the diversity of marine *Arthrinium* from Korea. In this study, a total of seven cultures were collected from marine brown algae *Sargassum* sp. and identified by phylogenetic analysis [4]. They were classified into four clades. Among them, two clades were identified with *A. arundinis* and *A. saccharicola*, respectively. These *Arthrinium* species are described based on macro- and micro-scopic observation. A phylogenetic tree of *Arthrinium* species based on translation elongation factor 1-alpha (EF1) and beta-tubulin (TUB) sequences was also offered.

Kor. J. Mycol. 2016 December, **44**(4): 259-262
https://doi.org/10.4489/KJM.2016.44.4.259
pISSN 0253-651X • eISSN 2383-5249

***Corresponding author**
E-mail: jae-jinkim@korea.ac.kr

Received November 7, 2016
Revised December 2, 2016
Accepted December 13, 2016

Materials and Methods

Arthrinium Cultures and analysis of phenotype

Arthrinium cultures were collected previously [4]. They were obtained from the Korea University Culture Collection (KUC). Among them, three strains, KUC21221, KUC 21229 and KUC21261, were analyzed to be new to Korea. Cultures were grown on 9 cm-diam plastic petri dishes contained 20 mL of oatmeal agar (OA; oatmeal agar 72.5 g, DW 1 L; Difco, Detroit, MI, USA), potato dextrose agar (PDA; potato dextrose agar 39 g, DW 1 L; Difco) or malt extract agar (MEA; malt extract 20 g, agar 15 g, DW 1 L; Difco). The cultures were grown in room temperature. After a week, colonies were observed and pictures were taken using NEX-5R digital camera (Sony, Tokyo, Japan). Observation of colonies had been performed

until two weeks grown.

Morphological analyses of microscopic characters were observed with Olympus BX51 light microscope (Olympus, Tokyo, Japan). Pictures of conidiophore and conidia were taken using the same microscope mounted with Olympus DP20 microscopic camera (Olympus). Measurements were made from DW mounts. At least 30 units of each parameter were measured for each collection. To make reliable data, 5% of the measurements from each end of the range were removed. The isolates were deposited at the National institute of Biological Resources (NIBR), Incheon, South Korea.

Phylogenetic analysis

Genomic DNAs of *Arthrinium* spp. from KUC were previously extracted [4]. Genomic DNAs extraction from cultures was performed using Accuprep Genomic DNA extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer's protocol. PCR reaction of EF1 region was carried out according to the previously described method [6]. DNA sequencing was performed using Sanger method with 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) by Macrogen (Seoul, Korea). Sequences of TUB region were obtained by previous study [4].

The obtained EF1 and TUB sequences were proofread and aligned with the selected reference sequences from GenBank using MAFFT 7.130 [7], and modified manually with MacClade 4.08 [8], respectively. Both EF1 and TUB datasets contained 588 characters. Datasets were tested by MrModeltest 2.3 using the AIC criteria with default options [9], respectively. The HKY+I+G model or GTR+I+G model were chosen for EF1 or TUB dataset, respectively, under the AIC criteria as a result of the test. Bayesian analysis was performed with MrBayes 3.2.1 [10]. Phylogenetic tree was created according to previous study [4].

Results and Discussion

Taxonomy

***Arthrinium arundinis* (Corda) Dyko & B. Sutton, Mycotaxon 8: 119 (1979) (Fig. 1A~1D)**

Teleomorphic synonym: *Apiospora montagnei* Sacc.

Colonies flat, spreading, aerial mycelium abundant, white to grey colored. On MEA, conidia forming in abundant, scattered; no distinctive odor or diffusing pigment. On PDA and OA, aerial mycelium abundant and cottony. Mycelium consisting of smooth, hyaline, branched, septate, hyphae measured 1.5~4 μm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, pale brown, smooth, ampulliform, 5~12 × 2~4 μm, apical neck 3~5 μm long, basal part 4~5.5 μm long. Conidia brown, smooth, globose, 5.5~7.5 × (4~)5~6 μm.

Note: This species is easily isolated from bamboos, such as *Arundinaria* spp. like its epithet: '*arundinis*' [1]. Our strains and other *A. arundinis* were monophyletic with a high posterior probability (100%, Fig. 2).

Cultures examined: KOREA, Jeju Island, N33°23'39.3" E126°14'23.2", isolated from *Sargacium* sp., 10 January 2015, Seokyoon Jang (Culture KUC21229, GenBank KT 207645; KUC21261, GenBank KT207677).

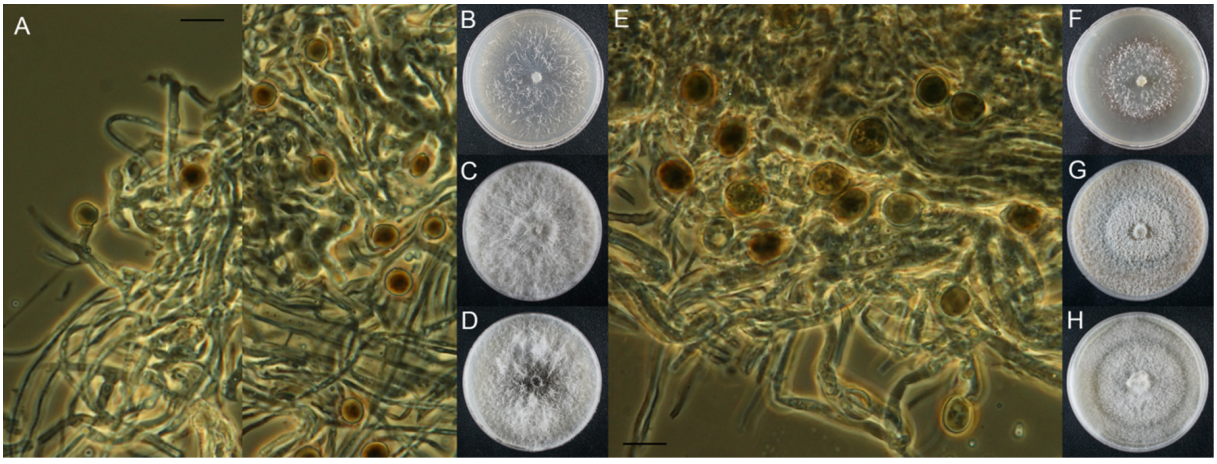

Fig. 1. *Arthrinium arundinis* (A~D) and *Arthrinium saccharicola* (E~H). A, conidiogenous cells and conidia; B, colonies on MEA; C, colonies on OA; D, colonies on PDA; E, conidiogenous cells and conidia; F, colonies on MEA; G, colonies on OA; H, colonies on PDA. MEA, malt extract agar; OA, oatmeal agar; PDA, potato dextrose agar (scale bars = 10 μm).

***Arthrinium saccharicola* F. Stevens, J. Dept. Agric. Porto Rico 1(4): 223 (1917) (Fig. 1E~1H)**

Colonies flat, spreading, with sparse aerial mycelium, conidia forming in few, white to grey colored with dark brown patches on agar media. On OA and PDA at first white colored, sometimes becoming pinkish; brown pigment diffused in media. Mycelium consisting of smooth, hyaline, branched, septate, hyphae measured 2.5~4 μm diam. Conidiogenous cells forming rare, aggregated in clusters on hyphae, ampulliform, 6~10 × 2.5~4 μm, apical neck 2~4 μm long, basal part 3~5 μm long. Conidia brown, smooth, granular, globose to ellipsoid, (7.5)8~9.5 (~10) × (7~)7.5~8(~8.5) μm.

Note: KUC21221 showed high activities of cellulolytic enzymes, antifungal metabolites and antioxidant capacity [4]. This strain often altered irregular form which grows slowly. KUC21221 and other *A. saccharicola* were monophyletic with high support (100% of PP, Fig. 2).

Culture examined: KOREA, Jeju Island, N33°23'39.3" E126°14'23.2", isolated from *Sargacium* sp. 10 January 2015, Seokyoon Jang (culture KUC21221, GenBank KT 207637).

Phylogenetic analysis

Some species of *Arthrinium* formed species complexes in a phylogenetic analysis based on internal translated spacer (ITS) or 28s ribosomal RNA, large subunit (LSU) region [3]. To avoid this problem, EF1 and/or TUB with higher resolution power were recommended [3, 4]. *Arthrinium* cultures examined in this study were classified into

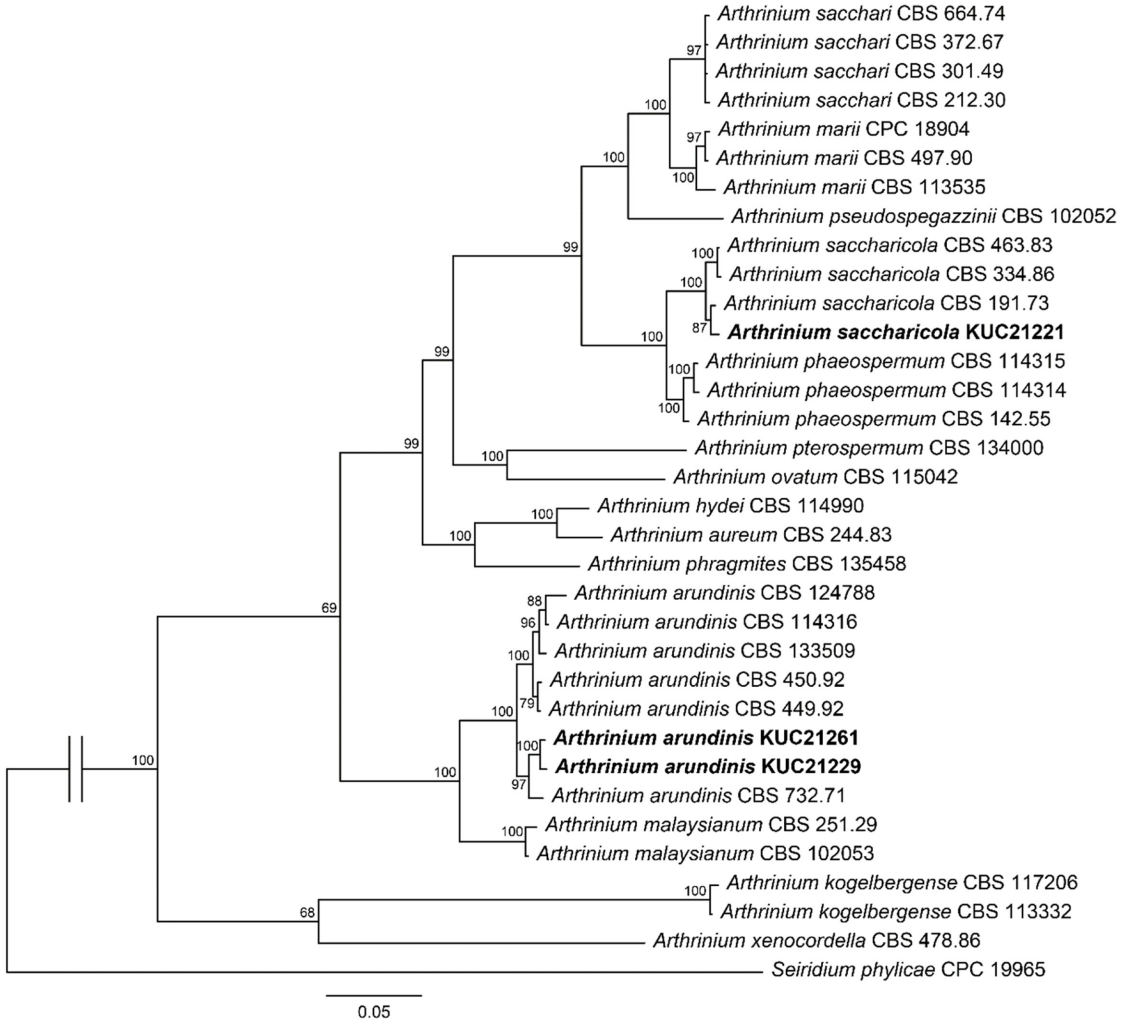

Fig. 2. The 50% majority-rule consensus tree which contains 34 taxa and 1,153 characters obtained from the Bayesian analysis based on combined translation elongation factor 1-α (EF1), and β-tubulin (TUB) sequence alignment. Numbers above branches indicate posterior probabilities. Fungal cultures examined in this study are in bold. The scale bar indicates nucleotide substitutions per position.

two clades. Morphology of the tree was in line with other studies [3, 4]. Three cultures were identified with *A. arundinis* or A. *saccharicola*. We suppose more than 10 species of *Arthrinium* present in Korea. Bamboos and marine environments are major habitats of *Arthrinium* [1-4]. Thus, further study with fungi from these habitats is needed to reveal bio-diversity of Korean *Arthrinium*.

Acknowledgements

This work was also supported by the Project on Survey and Discovery of Indigenous Species of Korea funded by NIBR of the Ministry of Environment (MOE), Republic of Korea. We thank Mr. Seongpil An of Solar cell & aerosol laboratory (Korea University, Seoul, Korea), who collected brown algae from Jeju Island.

REFERENCES

1. Kim JJ, Lee SS, Ra JB, Lee H, Huh N, Kim GH. Fungi associated with bamboo and their decay capabilities. Holzforschung 2011;65:271-5.
2. Suryanarayanan TS. Fungal endosymbionts of seaweeds. In: Raghukumar C, editor. Biology of marine fungi. Berlin: Springer; 2012. p. 53-69.
3. Crous PW, Groenewald JZ, A phylogenetic re-evaluation of *Arthrinium*. IMA Fungus 2013;4:133-54.
4. Hong JH, Jang S, Heo YM, Min M, Lee H, Lee YM, Lee H, Kim JJ. Investigation of marine-derived fungal diversity and their exploitable biological activities. Mar Drugs 2015;13:4137-55.
5. Khan SA, Hamayun M, Kim HY, Yoon HJ, Seo JC, Choo YS, Lee IJ, Kim SD, Rhee IK, Kim JG. A new strain of *Arthrinium phaeospermum* isolated from *Carex kobomugi* Ohwi is capable of gibberellin production. Biotechnol Lett 2009;31:283-7.
6. Huh N, Jang Y, Lee J, Kim GH, Kim JJ. Phylogenetic analysis of major molds inhabiting woods and their discoloration characteristics. Part 1. Genus *Trichoderma*. Holzforschung 2011; 65:257-63.
7. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 2013;30:772-80.
8. Maddison DR, Maddison WP. MacClade 4: analysis of phylogeny and character evolution. Version 4.08a. Sunderland: Sinauer Associates; 2005.
9. Nylander JA. MrModeltest v2. Uppsala: Evolutionary Biology Centre, Uppsala University; 2004.
10. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 2003;19:1572-4.