RESEARCH ARTICLE

First Report of *Allantophomopsiella pseudotsugae* Isolated from Soil in Korea

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ABSTRACT

A fungal isolate designated 17E029 was isolated from a soil sample in Jeju, Korea. The strain was similar to other *Allantophomopsiella* species in its morphological characteristics such as grey mycelia, conidiophore, and conidia sizes. The isolate produced aerial mycelia, which appeared grey on the reverse side of the media surfaces and turned black on the front side of the colonies. The conidiophores emanating from the hyphae were hyaline, grey, aseptate, branched, and 6.7~9.2 × 1.8~2.5 µm. Conidiogenous cells were ovoid to subcylindrical, discrete, guttulate, and hyaline. Conidia were hyaline, aseptate, smooth, guttulate, oval to subcylindrical, irregular in shape, and 6.0~7.8 × 3.0~3.4 µm. The strain was confirmed based on phylogenetic analysis of the closest related organism, *A. pseudotsugae* CBS 288.37, using the partial 28S, internal transcribed spacer rDNA regions, and partial RNA polymerase II second largest subunit locus (*RPB2*) gene sequences along with its culture characteristics. Therefore, morphological observations and phylogenetic analysis revealed that strain 17E029 is similar to the previously identified *A. pseudotsugae*. Hence, this species was described as *A. pseudotsugae* strain 17E029, which is a new record in Korea.

Keywords: *Allantophomopsiella pseudotsugae*, Phacidiaceae, Soil-inhabiting fungi

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INTRODUCTION

Allantophomopsiella was recently reported and accommodated with the type species *A. pseudotsugae*, a pathogen of conifers, named for its morphological similarity to the genus *Allantophomopsis* [1]. *A. pseudotsugae* was reported only in European countries such as Germany, UK, Netherlands and Norway, and mostly isolated from *Pinus* spp. [1] and it has several synonymic name, such as *Phomopsis pseudotsugae*, *Allantophomopsis pseudotsugae*, *Phacidiella coniferarum*, *Potebniamyces coniferarum*, *Phacidiopycnis pseudotsugae*, and *Phacidium coniferarum*, which were recently combined and reported as *A. pseudotsugae* [1,2]. Meanwhile, *Phomopsis pseudotsugae* (=*A. pseudotsugae*), a species of *Phomopsis*, was found to cause *Phomopsis* disease in conifers by attacking the young shoots of the plants [3]. According to Sharma & Snowdon [4,5], *Potebniamyces*

pyri, a species of genus *Potebniamyces* that causes *Phacidiopycnis* rot, was previously reported only in Europe and India, although the epidemiology of *Phacidiopycnis* rot in these production regions is unclear. *Phacidiopycnis* rot is an important cause of storage decay in 'd'Anjou pear' fruit, the main winter pear variety grown in the USA [6].

In this study, different fungal strains were isolated during the observation of unreported fungal species in Korea. Based on its morphological characteristics and results of molecular analysis, the isolated fungus was an undescribed species belonging to the genus *Allantophomopsiella*. Here, this fungus is identified and illustrated as an unreported species in Korea.

MATERIALS AND METHODS

Sampling and fungal strain isolation

Fungal isolate 17E029 used in this study was collected from a soil sample in Jeju, Korea (33°24'05.3"N, 126°40'31.3"E). The soil sample was collected from the ground, air-dried, and stored in a plastic bag at 4°C. A conventional dilution planting technique was applied to isolate the fungus [7]. One gram of soil sample was mixed with 10 mL of sterile distilled water, and the suspension was vortexed and diluted. Next, 2~3 drops of the suspension were spread onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) in petri dishes. To examine the growth rate of fungal colonies, the petri dishes were incubated at 25°C for 5 days. Individual colonies were purified by transferring them onto fresh media, such as PDA, malt extract agar (MEA; Difco, Detroit, MI, USA), and oatmeal agar (OA; Difco, Detroit, MI, USA), and then incubated again at 25°C for 14 days until mycelium had grown. The pure cultures were stored on PDA slants at 4°C until use.

Morphological characterization

Colony morphology and conidia characteristics were observed, measured, and photographed after 14 days of incubation. Images were acquired under a light microscope (BX-50, Olympus, Tokyo, Japan).

DNA extraction, PCR, sequencing and phylogenetic analysis

Total genomic DNA from strain 17E029 was extracted from the fungal mycelia grown on the PDA plate using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) following the manufacturer's protocol. The primers LSU1Fd [8] and LR5 [9] were used to amplify the partial 28S rDNA. The ITS1F/ITS4 primer pair was used to amplify the internal transcribed spacer (ITS) region of the nuclear rRNA gene [10]. fRPB2-5F and fRPB2-7cR primers were used to amplify the partial RNA polymerase II second largest subunit locus (*RPB2*) [11]. Amplifications were performed in a PCR machine (Applied Biosystems, Foster City, CA, USA). The amplified PCR fragments were purified using ExoSAP-IT (USB Corp. Cleveland, OH, USA). The relationships between the attained sequences were analysed using BLAST from NCBI and GENETYX-WIN (ver. 3.2) program. The 503, 744 and 1,087 base pairs for ITS, LSU and *RPB2*, respectively, were obtained from novel sequences after depositing the sequences in NCBI GenBank under accession numbers LC434625,

LC434626 and LC434630. Reference sequences were obtained from GenBank under the accession numbers indicated in Table 1. Alignment and phylogenetic tree construction was conducted by the maximum parsimony method using MEGA 6 software [12] with bootstrap analysis of 1,000 replications.

RESULTS AND DISCUSSION

Morphological and phenotypic characteristics of isolate 17E029

Taxonomic descriptions and microphotographs of morphological structures of the isolate 17E029 are shown in Table 2 and Figure 1. Colonies grew moderately, reaching 39 mm in diameter on PDA, 32 mm on OA and 29 mm on MEA at 25°C after 14 days of incubation. In all growth media, colonies

Table 1. GenBank codes of fungal strains used for phylogenetic analyses in this study.

Species	GenBank Accession Numbers			
	Strain no.	LSU	ITS	*RPB2*
Allantophomopsiella pseudotsugae	CBS 288.37	KJ663863	KY484687	KT389569
A. pseudotsugae	17E029	LC434626	LC434625	LC434630
Allantophomopsis cytisporea	CBS 262.85	KJ663869	KJ663830	KJ663910
Allantophomopsis sp.	CBS 109.22	KJ663861	KJ663822	KJ663902
Allantophomopsis sp.	CBS 322.36	KJ663880	KJ663839	KJ663921
Bulgaria inquinans	CBS 118.31	KJ663870	KJ663831	KJ663911
Phadium fennicum	CBS 457.83	KJ663881	KJ663840	KJ663922
P. lauri	CBS 198.68	KJ663890	KJ663849	KJ663930
P. lacerum	CBS 338.70	KJ663883	KJ663842	KJ663924
P. vaccinii	CBS 444.71	KJ663896	KJ663855	KJ663936
Potebniamyces pyri	CBS 282.55	KJ663862	KJ663823	KJ663903
P. pyri	CBS 322.63	KJ663900	KJ663859	KJ663940
Sarcotrochila longispora	CBS 273.74	KJ663877	KJ663836	KJ663918

Table 2. Morphological characteristics of isolate 17E029 compared to the closest species *Allantophomopsiella pseudotsugae*

Characteristics	Isolate 17E029 *	*Allantophomopsiella pseudotsugae*[a]
Cultural characteristics	Mycelia appeared grey on reverse side of the media surfaces and turned black on front side of colonies.	Mycelia are reverse iron-grey, and olivaceous grey with spots of iron-grey.
	Colonies moderately growing, attaining 39 mm in diameter on PDA, 32 mm on OA, and 29 mm on MEA at 25°C after 14 days of incubation.	N/A
Conidiophore size (μm)	6.7~9.2 × 1.8~2.5	5.0~15.0 × 2.5~3.5
Position and shape	Conidiophores emanating from hyphae were hyaline or grey, aseptate, branched.	Conidiophores arising from inner layer of conidioma, while decreased to conidiogenous cells, septate, branched.
Conidia size (μm)	6.0~7.8 × 3.0~3.4	4.0~7.0 × 2.0~3.0
Position and shape	Conidia were hyaline, smooth, aseptate, guttulate, oval.	Conidia are hyaline, smooth, aseptate, guttulate, ellipsoid to fusiform.

N/A = not available in previous reference, *Fungal strain studied in this paper,
[a]Source of description (Crous et al., 2014)

of the isolate 17E029 first appeared as light white coloured with few aerial mycelia and then changed to grey on the reverse side of the media surfaces, showing feathery irregular margins (Figure 2A~2F). On the front side of the PDA and MEA surfaces, the mycelia turned black; this colour emanated from the central part of the colonies (Figure 1A, 1E). Sporulation of the 17E029 isolate occurred on PDA media. Cnidiophores arising from hyphae were hyaline or sometimes grey, aseptate, branched, and 6.7~9.2 × 1.8~2.5 μm (Fig. 2A). Conidiogenous cells were discrete, ovoid to subcylindrical, guttulate, and hyaline (Fig. 2B). Conidia were observed as oval to somewhat subcylindrical, hyaline, aseptate, smooth, guttulate, irregular in shape, and 6.0~7.8 × 3.0~3.4 μm (Fig. 2C). The morphology of strain 17E029 was compared to previous descriptions of the closest species *Allantophomopsiella pseudotsugae* [1] in Table 2.

As currently described, *A. pseudotsugae* CBS 321.53 grew with colonies dispersing, uniform with scarce aerial mycelium, and feathery margins. The PDA surface was olivaceous grey and the reverse side was iron-grey. On the OA surface, the mycelium appeared as olivaceous grey with some spots of iron-grey. Conidiophores emanated from the inner layer of the conidioma, while decreased to

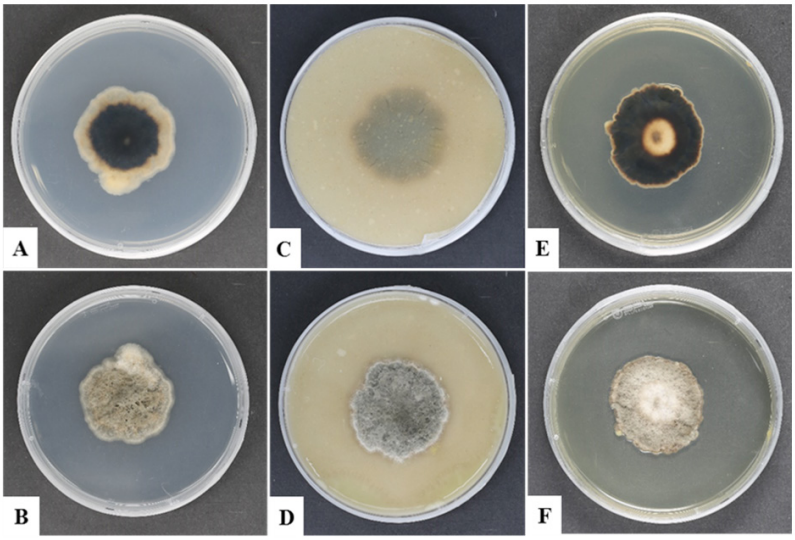

Fig. 1. Cultural characteristics of 17E029 (A~F), Colony on PDA (A, B), OA (C, D), and MEA (E, F) showing front and reverse sides.

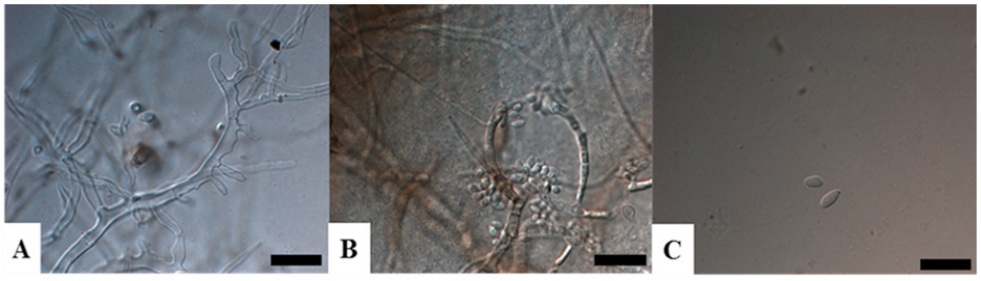

Fig. 2. Morphological characteristics of 17E029. A, Conidiophores; B, Conidiogenous cells; C, Conidia; Scale bars = 10 μm.

conidiogenous cells, septate, branched, and 5.0~15.0 × 2.5~3.5 μm. The conidia are hyaline, smooth, aseptate, guttulate, ellipsoid to fusiform, 4.0~7.0 × 2.0~3.0 μm, bearing mucoid apical appendages (can only be seen in water), and flabelliform to irregular in shape [1]. Based on the morphological characteristics and phylogenetic analysis, strain 17E029 was matched with previously described *A. pseudotsugae* CBS 288.37. Thus, the species was described as a new record of *A. pseudotsugae* in Korea. The fungal isolate 17E029 was deposited in the National Institute of Biological Resources (NIBRFG0000502441).

Molecular phylogeny of isolate 17E029

The identity of isolate 17E029 was confirmed by BLAST analysis, which revealed 100% similarity to the partial 28S rDNA sequence of *A. pseudotsugae* MH871973 and 99% similarity of *Phacidium lauri* MH871978, 99% similarity to the ITS region sequence of *A. pseudotsugae* MH857222 and 99% similarity to *P. pyri* MF375775, 90% and 88% similarities to the partial *RPB*2 sequence of *P. pyri* DQ470900 and *Allantophomopsis* sp. KY676741, respectively. Based on the phylogenetic tree, the representative isolate 17E029 was placed in the clade containing the reference isolate *A. pseudotsugae* CBS 288.37 with a bootstrap value of 100% in the phylogenetic tree constructed based on a concatenated alignment of the LSU, ITS, and *RPB*2 sequences (Fig. 3). The phylogenetic analysis results strongly support the fact that the isolate 17E029 is *A. pseudotsugae*, a new record in Korea.

The generic name *Allantophomopsiella* was introduced to accommodate *A. pseudotsugae*, a pathogen of conifers [1]. Many species in this genus and their synonymous genera are fungi that

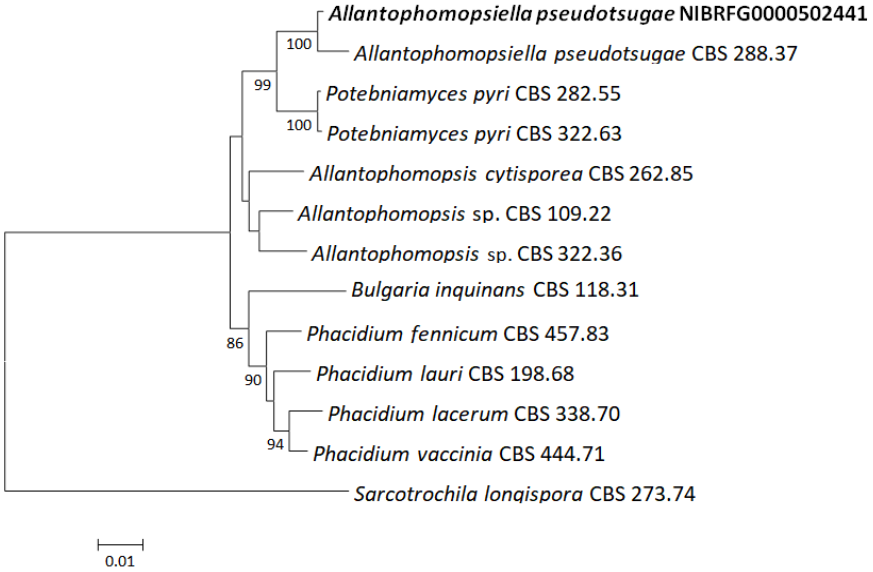

Fig. 3. Neighbour-joining tree based on obtained sequences of the internal transcribed spacer rDNA region, LSU, and partial RNA polymerase II second largest subunit locus (*RPB*2). *Sarcotrochila longispora* strain CBS 273.74 was an outgroup. The fungal strain which examined in this study was indicated in bold and the bootstrap value below 70% is not shown. The scale bar, 0.01 indicates the number of nucleotide substitutions.

damage conifers such as *A. pseudotsugae* was found to cause serious damage to larch in Iceland [13]. And also, *A. pseudotsugae* was described to cause *Phomopsis* disease in conifers, which involves attack on the young plant shoots [3]. Moreover, *A. pseudotsugae* were found from the *Pinus* wood in Germany, needles of *Pinus sylvestris* in Netherlands and Norway, and also from 30-yrs-old *Picea abies* as a dieback disease agent in UK [1]. The cultural and morphological characteristics along with their molecular analysis results confirmed our isolate 17E029 as *A. pseudotsugae* which is a new record in Korea. So, further investigation is necessary to explore the etiology of *A. pseudotsugae* as well as the pathogenicity based on Korean ecological and environmental conditions.

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