

Identification of *Acremonium acutatum* and *Trichothecium roseum* isolated from Grape with White Stain Symptom in Korea

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Abstract During 2010 and 2012 grape harvest seasons in Gyeonggi-do, Korea, a white stain symptom was observed on the harvested grape fruits in ‘Campbell-Early’ and ‘Kyoho’ varieties. In samples collected from the infected vine, two different strains of pathogenic fungi have been found and identified as *Acremonium acutatum* and *Trichothecium roseum* based on fungal morphology and nucleotide sequence of internal transcribed spacer (ITS) and supported by the phylogenetic analysis of the rDNA-ITS region. The DNA homologies of the isolated strains were 99.8% and 99.6% identical with *T. roseum* (IFB-22133) and *A. acutatum* (CBS682.71), respectively. In the pathogenicity test, the spores of *A. acutatum* and *T. roseum* sprayed on the grapes caused white stain symptoms on the fruits in two weeks after the artificial inoculation, which is similar to observations in the field. To our knowledge, this is the first report of white stain symptoms caused by *A. acutatum* and *T. roseum* on the grapes in Korea.

Keywords *Acremonium acutatum*, Grape, *Trichothecium roseum*, White stain symptom

White stain symptom has been reported in the vineyards of South Korea since 1999, lowering the quality of grapes and causing serious economic losses. The fungus of white stain symptom can infect cane, internode and cluster of the grape-tree (Fig. 1). The presence of mycelia with conidiophores and conidia on the surface of the host tissue give it a white stain or powdery mildew appearance. The symptom occurred in several grape varieties, including ‘Campbell-Early’ and ‘Kyoho’. Although the symptom was severe, it seldom resulted in appreciable damage to vine health, except the dramatic reduction of grape quality. In previous reports, the white stain symptom was caused by

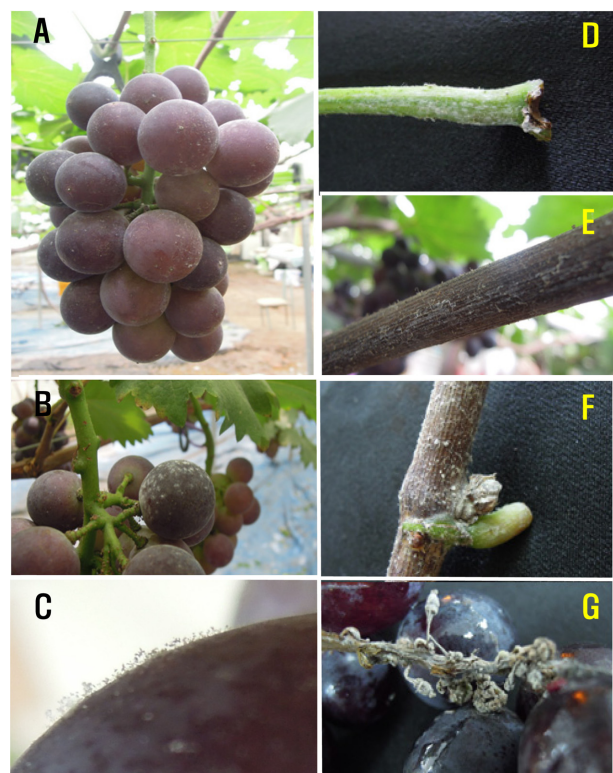


Fig. 1. Typical appearance of the white stain symptoms on the grape fruits (A~C), petiole (D), stem (E), axillary bud (F), and cluster (G).

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Acremonium sp., *Trichothecium* sp. [1] and *Hanseniaspora* sp. [2]. However, the exact identification of the causal pathogen has not yet been reported. In this study, we identified the causal pathogen associated with the white stain symptom on grapes in Gyeonggi province, Korea, based on cultural characteristics, pathogenicity, and internal transcribed spacer (ITS) sequence analysis.

MATERIALS AND METHODS

Pathogenic fungus collection and isolation. In order to identify pathogenic fungus, causing the white stain symptom over growing periods, an infected grape was collected from the orchards located Miyang-myeon (Anseong-si) for 'Kyoho' variety and Seoshin-myeon (Hwaseong-si) for 'Campbell-Early' variety in Gyeonggi-do in the harvest seasons from 2010 to 2012.

Infected fruits washed with sterile distilled water (SDW), 100 μ L of the wash water spread on water agar (WA). Once spread on WA, it was incubated in an incubator at 25°C, for three days. After three days, the marginal mycelium of single colony grown on WA was isolated and then incubated on potato dextrose agar (PDA) medium for additional 7 days. Isolates were used to identification and pathogenic test.

Identification of isolated fungus. Identification of isolated fungus was based on morphological characteristics as well as comparison nucleotide sequence of ITS region. Of the isolated fungi *Acremonium* spp. and *Trichothecium* spp. were found to be the most abundant one, thus the optimal incubation temperature of *Acremonium* spp. and *Trichothecium* spp. were investigated. Mycelium was cut with or by using a cork borer (No. 2, Daihan, Seoul, Korea) and then inoculation on the middle of PDA prior to sealing. *Acremonium* spp. and *Trichothecium* spp. were incubated for 20 days with 25°C and then the diameter of colony was measured. In addition, shape of mycelium, conidiophore and conidia observed using optical microscope

(de/mz16a; Leica, Wetzlar, Germany) in order to investigate morphological characteristics of fungal isolate. In order to compare nucleotide sequence of ITS region, DNA was extracted from freeze dried mycelium using the Nucleo spin soil kit (Macherey-Nagel, Dueren, Germany). Sequence of 16S rDNA gene was amplified using ITS1 (5'-CGTAG-GTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATT-GATATGC-3') primers [3]. Polymerase chain reaction (PCR) analysis was performed according to the method of Staats *et al.* [3]. For the PCR conditions of ITS1/ITS4, samples were denaturated at 94°C for 2 min followed by additional denaturation at 94°C for 40 sec. Sample annealing was done at 60°C for 1 min and then extended at 72°C for 1 min over 30 cycles. The final DNA synthesis condition was 72°C for five minutes. Amplified products of PCR were applied into 1.2% agarose gel electrophoresis and then nucleotide sequence was determined. Taxonomic relations between isolated fungi were analyzed using Kimua's 2-parameter distance of neighbor-joining method.

Pathogenicity test. Grape were artificially inoculated with a conidial suspension (1×10^6 conidia/mL) of the pathogen obtained from SDW. After inoculation, the fruits incubated for 20 days with 25°C. Two wk after the inoculation, the fungus was re-isolated from the symptoms of white stain.

RESULTS

Isolation and characterization of fungi. An epidemic of the white stain symptom on clusters and canes of the grape by dust-like particles occurred in many vineyards in Gyeonggi-do area in the harvest seasons. Two different pathogenic fungi have been collected from infected grape and identified as *A. acutatum* and *T. roseum* based on fungal morphology (Fig. 2). *Acremonium* sp., *Penicillium* sp., *Trichothecium* sp., *Cladosporium* sp., and *Aspergillus* sp. were separated from the rind of grape fruit, isolation frequency was 29%, 25%, 20%, 12%, and 2%, respectively.

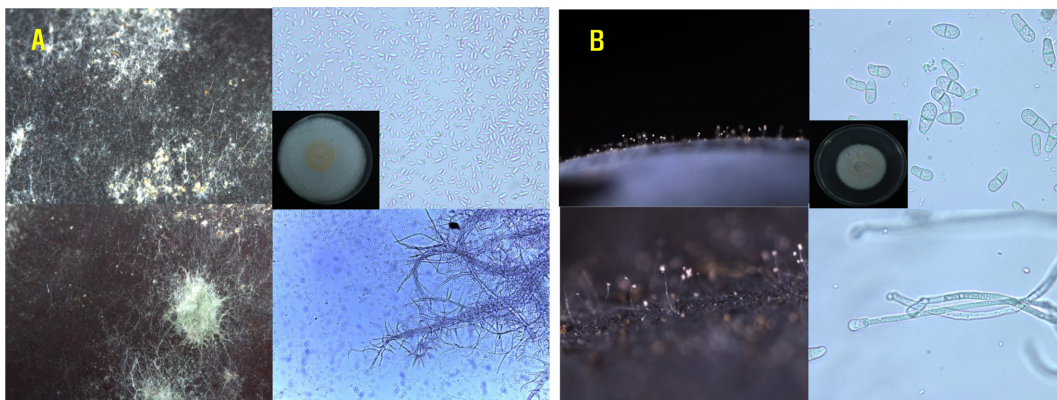


Fig. 2. Morphological characteristics of *Acremonium acutatum* (A) and *Trichothecium roseum* (B) isolated from the infected grapes.

Result of the pathogenicity tests, typical symptoms was reproduced when inoculated with a spore suspension of *Acremonium* sp. and *Trichothecium* sp.. However, both *Penicillium* sp. and *Cladosporium* sp. were not the major

causal pathogen of white stain symptom because blue and green mold occurred on the rind of the grape fruit when inoculated with a spore suspension of them. Colonies of *A. acutatum* reached 10~16 mm diameter on PDA in 10 days

Table 1. Comparison of morphological characteristics of the fungus isolated from grape those of *Acremonium acutatum* described previously

Characteristics		HKNU 004	<i>Acremonium acutatum</i> ^a
Colony Color		White to slightly ochraceous, granulose in the center	White to slightly ochraceous, granulose in the center
Phialide Shape		Slenderly awl	Slenderly awl
	Length (μm)	20~40	20~40
Conidia Shape		One cell, fusiform	One cell, fusiform
	Diameter (μm)	4~5	4~5

^aDescribed by Williams [4].

Table 2. Comparison of morphological characteristics of the fungus isolated from grape those of *Trichothesium roseum* described previously

Characteristics		HKNU 005	<i>Trichothesium roseum</i> ^a
Colony Color		Pale roseae	Pale roseae
Conidiophores Shape		Simple or branched below	Simple or branched below
	Length (μm)	150~260	140~280
Conidia Shape		Ellipsoidal, 2 cell	Ellipsoidal, 2 cell
	Diameter (μm)	18~22 × 8~10	16~20 (~25) × (7.5) 8~10 (~10.5)

^aDescribed by Gobayashi *et al.* [5] and Ishikawa *et al.* [6].

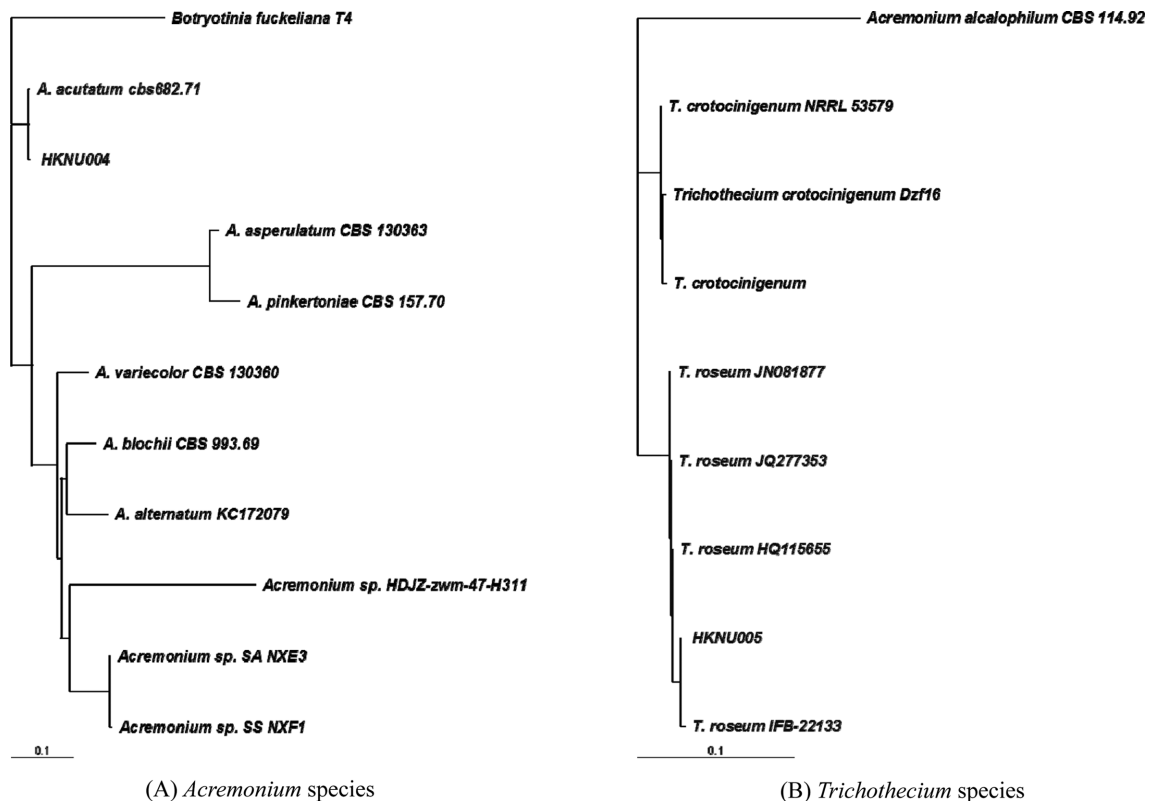


Fig. 3. Phylogenetic tree based on internal transcribed spacer sequences showing relationships among HKNU isolates from grape and closely relative *Acremonium* species (A) and *Trichothecium* species (B). The tree was constructed with the neighbor-joining algorithm using

at room temperature and had a white to pale pink color with granulose appearance in the center. Conidia were single cell, smooth-walled, and 4~5 µm in diameter (Table 1) [4]. Conidia of *T. roseum* were hyaline or brightly colored, two-celled, smooth-walled, 18~22 × 8~10 µm in size, of ovoid or ellipsoid shape, and characteristically held together in zigzag chains. Conidiophore was long (150~260 µm), slender, simple, septate, and bearing conidia meristem arthrospores apically-singly when young and successively after a slight growth of conidiophore apex (Table 2). Colony characteristics were taken from cultures grown on PDA in darkness at 25°C for 20 days. The measurements and taxonomic characteristics agreed with those of *T. roseum* (Pers.) Link ex Gray [5, 6].

Phylogenetic analysis. The neighbor-joining analysis showed that the ITS sequences of the isolates were similar to those of *A. acutatum* and *T. roseum* (Fig. 3) [7, 8]. In the phylogenetic tree, the isolate (HKNU004) was placed within a clade comprising reference isolates of *A. acutatum*. The DNA homology of HKNU004 was 99.8% identical with *A. acutatum* (CBS682.71). The other isolate (HKNU005) was placed within a clade comprising reference isolates of *T. roseum*. The DNA homology of HKNU005 was 99.6% identical with *T. roseum* (IFB-22133).

Pathogenicity test. Same disease symptoms as in the field were reproduced on the fruits at two weeks following the artificial inoculation, and the same fungus were re-isolated from the symptoms, confirming that *A. acutatum* and *T. roseum* were related to the white stain symptom on the grape surface (Fig. 4). Disease development started on the seventh day, extensive mycelial development and sporulation of *A. acutatum* and *T. roseum* were observed. At the end of 20 days, typical symptoms with white stain were appeared on the pedicel, rachis and fruits of infected grape clusters. When observed under a stereoscopic microscope, infected grape fruit by *Acremonium* sp. was characteristically covered with pale pink colored mass of

conidia and white colored dusty or powdery appearance of mycelium. On the other hand, infected grape fruit by *Trichothecium* sp. was covered with white colored powdery appearance of mycelium.

DISCUSSION

The white stain symptom observed in the vineyards of Gyeonggi-do in Korea, with typical dust-like particles appearing on the grape vine, axillary bud, petiole and cluster of infected plants was similar to powdery mildew, but the diseases affected the plants quite differently. The observed white stain symptom only covered the surface of the grape, including fruit, leaf and branch, but does not break into the tissue of the grape fruit. The hyphae are present on the surface of the grapes and are easy to remove by water or hands. Nevertheless, the contaminated fruits are difficult to sell. Based on mycological characteristics, pathogenicity test, and the ITS sequence analysis, the causal fungus were identified as *A. acutatum* and *T. roseum*. The cause of similar symptoms observed in Gimcheon province was due to *Hanseniaspora* sp. and the infection was adversely affecting wine fermentation [1]. *Acremonium* species have been known as mycoparasites against plant pathogenic fungi such as *Aspergillus*, *Alternaria*, etc. [9]. The genus *Acremonium* contains about 100 species, of which most are saprophytic, being isolated from dead plant material and soil [10]. It was reported that *Acremonium strictum* was the causal pathogenic fungus in strawberry [11]. However, plant disease caused by *A. acutatum* has not been reported in Korea. *T. roseum* has been reported as a causal agents for pink mold rot on unishiu orange [12], pears [13], matured melon [14] and tomato [15] in Korea.

According to the results of this study, the cause of the disease in Gyeonggi province was *A. acutatum* and *T. roseum*, and the symptoms are different as well: the white hyphae are observed visually on the surface of the infected fruit and stem. To our knowledge, this is the first report of white stain symptoms caused by *A. acutatum* and *T.*

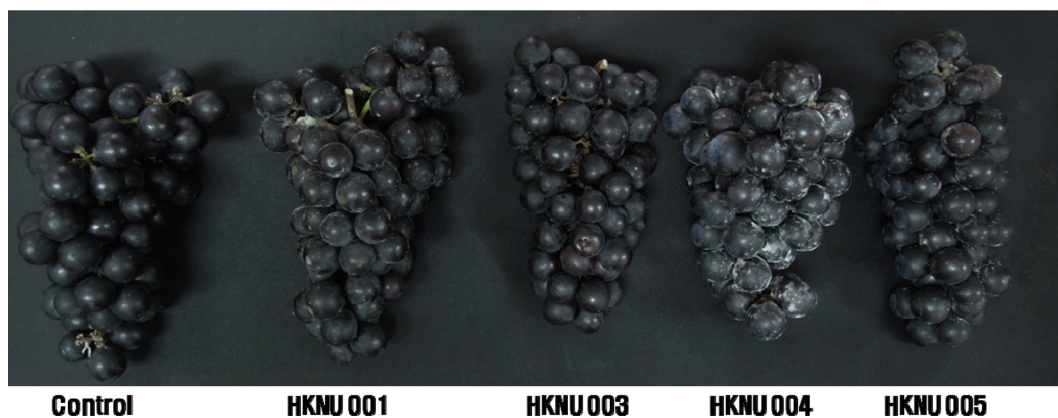


Fig. 4. Symptoms of white stain symptom on the grape fruits artificially infected with isolates (HKNU001, *Cladosporium* sp.; HKNU003, *Penicillium* sp.; HKNU004, *Acremonium acutatum*; HKNU005, *Trichothecium roseum*).

roseum in Korea.

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