Mycobiology 39(2) : 140-142 (2011)
© The Korean Society of Mycology

DOI:10.4489/MYCO.2011.39.2.140
pISSN 1229-8093
eISSN 2092-9323

First Report of *Rhizopus oryzae* as a Postharvest Pathogen of Apple in Korea

Jin-Hyeuk Kwon[1]*, Jinwoo Kim[2] and Won-Il Kim[3]

[1]*Gyeongsangnam-do Agricultural Research and Extension Services, Jinju 660-360, Korea*
[2]*Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Korea*
[3]*National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Korea*

(Received January 24, 2011. Accepted May 13, 2011)

Soft rot in apple caused by *Rhizopus oryzae* was found for the first time in Korea. A detailed description of the specimen is given along with its internal transcribed spacer rDNA sequence. The fungus was identified as *Rhizopus oryzae* based on the mycological characteristics, molecular data, and pathogenicity testing.

KEYWORDS : Postharvest disease, *Rhizopus oryzae,* Soft rot

Postharvest diseases including soft rot occur on the succulent tissues of vegetables, fruits, and ornamental plants worldwide. Postharvest losses as a result of fungal infection occur if products are stored at the incorrect temperature, stored for an extended period of time at cold temperatures, or as a result of mechanical failure during storage or transport [1]. In March 2010, a disease suspected to be *Rhizopus* soft rot was observed on apple fruit at commercial markets in Jinju, Korea.

Symptoms. The first symptom of soft rot on apple fruit was a water-soaked appearance to the affected tissue. The diseased parts later disintegrated into a mushy mass of disorganized cells that sloughed off. Rapid softening and disintegration of the diseased tissue followed. White mycelia formed on infection sites of apples and gradually covered the fruit with tufted whisker-like gray sporangiophores and sporangia (Fig. 1A). Longitudinal sections of the infected apple fruit appeared softened and severely rotted (Fig. 1B).

Mycological characteristics. The causal fungus was isolated from the diseased fruit sampled from commercial markets. Sporangiospores, sporangia, and sporangiophores were observed under a light microscope (Table 1) [2]. The fungal colonies that grew on potato dextrose agar were initially white and cottony, then became heavily speckled with sporangia, and finally became brownish-grey to blackish-grey and spread rapidly with stolons fired at various points to the substrate by rhizoids (Fig. 2A). The optimum temperature for mycelial growth was 30°C, with good growth still apparent at 37°C. Sporangiospores were unequal, numerous, irregular, sub-globose or oval, angular with striations, and 4~8 μm (Fig. 2B). Sporangiophores were usually straight, mostly 8~20 μm, smooth-walled, simple or branched, non-septate, long, and arose from stolons opposite rhizoids usually in groups of 3~5 or more. Sporangia were globose, white at first, and then turned black with many spores, mostly 40~200 μm (Fig. 2C). Columella were globose to sub-globose, pale brown, and mostly 85~110 μm (Fig. 2D). Rhizoids and stolons were dark brown (Fig. 2E).

Pathogenicity testing. Twelve apple fruits were artificially inoculated with a representative fungus using the wound infection method. A conidial suspension (0.1 mL; 3×10^4 conidia/mL) of the causal fungus was placed on the surface of apple fruit. The inoculated fruit was kept in a moist chamber with 100% relative humidity at 30°C. After a 3 day incubation, the same fungal symptoms were reproduced: soft rot was observed on inoculated fruits that was identical to symptoms observed at the commercial markets (Fig. 1C and 1D). The causal pathogen was re-isolated from the lesions to prove Koch's postulates.

Internal transcribed spacer (ITS) sequence analysis. To confirm the identity of the causal fungus, the ITS rDNA of the isolate was amplified and sequenced using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 primers (5'-TCCTCCGCTTATTGATATGC-3'), as described by White *et al*. [3]. The resulting 626-bp sequence was deposited in GenBank (accession No. HQ897687). A phy-

*Corresponding author <E-mail : kwon825@korea.kr>

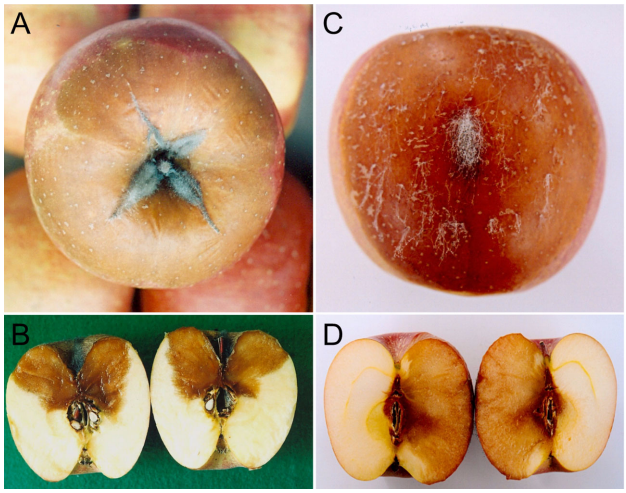

Fig. 1. Symptoms of soft rot on apple (*Malus pumila* var. *dulcissima* Koidz.) caused by *Rhizopus oryzae*. A, Soft rot symptoms on apple fruit sampled from commercial markets; B, Longitudinal section of apple. Symptoms were induced naturally; C, Symptoms induced by artificial inoculation; D, Longitudinal section of apple. Symptoms were induced artificially.

logenetic analysis was performed using MEGA4 with the neighbor-joining method and the Tajima-Nei distance model.

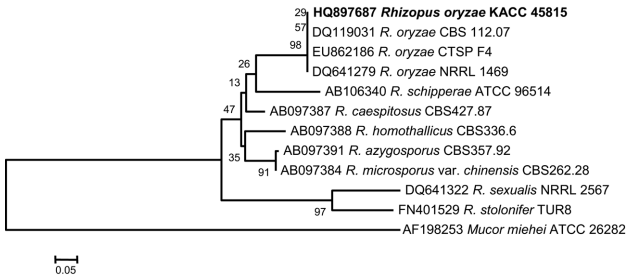

Fig. 3. Phylogenetic tree using internal transcribed spacer (ITS) sequences showing closest known relatives of *Rhizopus oryzae*, including soft rot fungus infecting *Malus pumila* var. *dulcissima* Koidz. DNA sequences from the NCBI nucleotide database were aligned using ClustalW, and a phylogenetic tree was constructed using the neighbor-joining method and visualized with TreeView. Numbers above the branches indicate bootstrap values. Bars indicate number of nucleotide substitutions per site. The present isolate infecting *M. pumila* is marked in bold.

Table 1. Comparison of morphological characteristics of soft rot fungus isolated from apple (*Malus pumila* var. *dulcissima* Koidz.) with previous descriptions of *Rhizopus oryzae*

Characteristics		Isolate in present study	*R. oryzae* [2]
Colony	Color	Brownish-grey to blackish-grey	Brownish-grey to blackish-grey
Sporangium	Shape	Globose	Globose
	Size	40~200 μm in diameter	30~210 μm in diameter
Sporangiospore	Shape	Sub-globose or oval	Sub-globose, limoniform
	Size	4~8 μm in length	4~10 μm in length
Sporangiophore	Size	8~20 μm in diameter	7~20 μm in diameter
Columellum	Shape	Globose to sub-globose	Globose to sub-globose
	Size	85~110 μm in diameter	90~120 μm in diameter

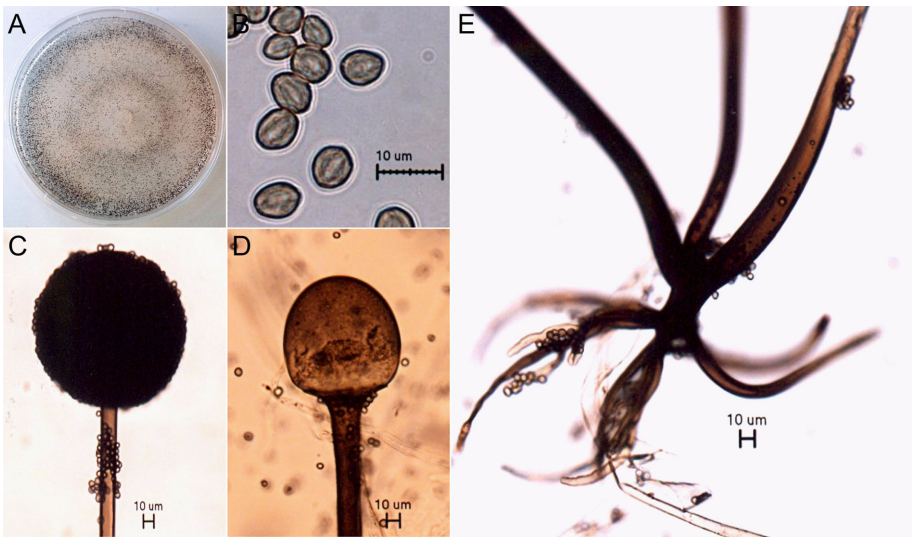

Fig. 2. Morphological characteristics of *Rhizopus oryzae* isolated from soft rot lesions on apple (*Malus pumila* var. *dulcissima* Koidz.). A, Colony on potato dextrose agar after a 7 day incubation; B, Sporangium and sporangiophore; C, Columella; D, Sporangiospores; E, Rhizoids.

Previously published ITS sequences from *R. oryzae* strains were included for reference, and *Mucor miehei* (GenBank accession No. AF198253) was used as an out-group [4]. In the phylogenetic tree, the present isolate was placed within a clade comprising *R. oryzae* references isolates (Fig. 3).

Soft rot of apple caused by *R. stolonifer* has been reported previously [5], but soft rot caused by *R. oryzae* has not been recorded in Korea [6]. The representative culture of the causal fungus was deposited in the Korean Agricultural Culture Collection (KACC 45815), National Academy of Agricultural Science, Suwon, Korea. Based on the mycological characters, molecular data, and pathogenicity testing of the host plant, the fungus was identified as *Rhizopus oryzae* Went & Prisen Geerligs [2]. This is the first report of *R. oryzae* on apple in Korea.

Acknowledgements

This work was conducted with the support of the Cooperative Research Program for Agriculture Science & Technology Development (PJ007345), Rural Development Administration, Korea.

References

1. Agrios GN. Plant pathology. 5th ed. New York: Academic Press; 2005.
2. Lunn JA. *Rhizopus oryzae*. CMI descriptions of pathogenic fungi and bacteria. No. 525. Kew: Commonwealth Mycological Institute; 1977.
3. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315-22.
4. Abe A, Oda Y, Asano K, Sone T. The molecular phylogeny of the genus *Rhizopus* based on rDNA sequences. Biosci Biotechnol Biochem 2006;70:2387-93.
5. Kwon JH, Jee HJ. Occurrence of rhizopus soft rot on apple fruit caused by *Rhizopus stolonifer* in Korea. Res Plant Dis 2008;14:57-60.
6. Korean Society of Plant Pathology. List of plant diseases in Korea. 5th ed. Seoul: Korean Society of Plant Pathology; 2009.