

## Soft Rot on *Cucumis melo* var. *makuwa* Caused by *Rhizopus oryzae*

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*Rhizopus oryzae* is reported for the first time on *Cucumis melo* L. var. *makuwa* Makino. A detailed description of this Korean specimen is given, along with its rDNA internal transcribed spacer sequence. On the basis of mycological characteristics and molecular data, the fungus was identified as *R. oryzae* Went & Prinsen Geerlig.

**KEYWORDS :** *Cucumis melo*, *Rhizopus oryzae*

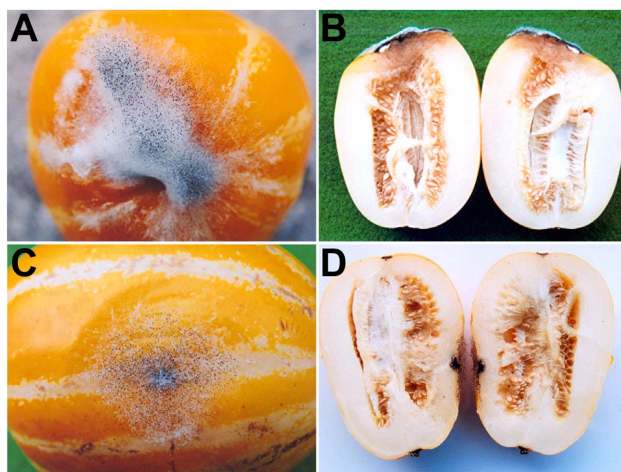
During the summer of 2009 and 2010, a disease suspected as *Rhizopus* soft rot occurred on oriental melon at the agricultural products wholesale market in Jinju. Only wounded mature fruits were affected; undamaged or immature melons were not attacked. Soft rot caused by *Rhizopus* sp. occurs on the succulent tissues of vegetables, fruits and ornamentals throughout the world. The disease mainly occurs on the market-shelf and during sale, transport, and storage [1].

**Symptoms.** The infected parts of the mature fruits appeared water-soaked at first, then became softened and

rotted rapidly. White mycelia grew from the primary infection and gradually covered the fruit with tufted whisker-like gray sporangiophores and sporangia (Fig. 1A). The infected tissues disintegrated into a watery rot. Longitudinal sections of the infected fruit appeared soft and severely rotted (Fig. 1B). The infection usually started from cracks that occurred at harvest.

**Mycological characteristics.** The causal organism was isolated from mycelial tips on the diseased fruits. Sporangia and sporangiophores were observed under the light microscope (Table 1). The colonies of fungus grown on potato dextrose agar were white and cottony at first, then became heavily speckled with the appearance of 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 25~30°C, with good growth still apparent at 37°C. Sporangiospores were unequal, numerous, irregular, sub-globose or oval, angular with striations, 6~10 µm in length (Fig. 2B). Sporangiophores were usually straight, mostly 10~20 µm in diameter, 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 60~200 µm (Fig. 2C). Columella were globose to sub-globose in shape, pale brown in color, and mostly 80~110 µm in diameter (Fig. 2D). Rhizoids and stolons were dark brown (Fig. 2E).

**Pathogenicity test.** Six fruits of Oriental melon were selected. One milliliter of the conidial suspension of *Rhizopus* ( $3 \times 10^4$  conidia/mL) was added dropwise to the wounded surface. The inoculated fruits were placed in a vinyl bag and kept in a humid chamber with 100% rela-

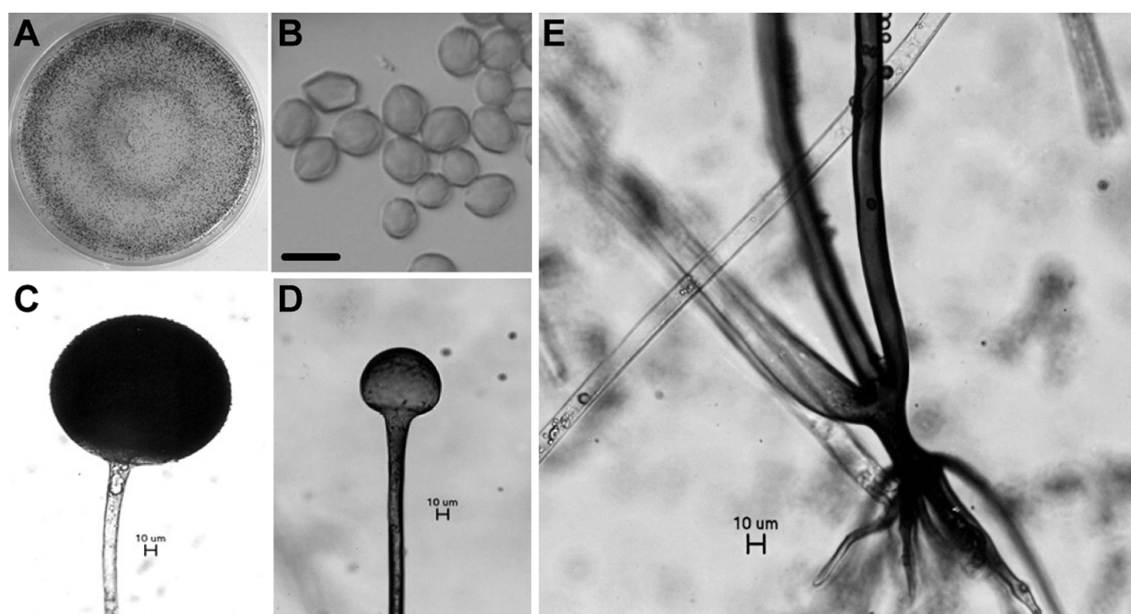


**Fig. 1.** Symptoms of rhizopus soft rot on oriental melon (*Cucumis melo*) caused by *Rhizopus oryzae*. A, Typical symptom showing water-soaking and formed mycelia, sporangia and sporangiospores on the surface of fruits; B, D, Longitudinal section of infected fruit; C, Symptom induced by artificial inoculation.

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**Table 1.** Comparison of morphological characteristics of the fungus isolated from oriental melon (*Cucumis melo* L. var. *makuwa* Makino) with the previous descriptions of *Rhizopus oryzae*

Characteristics	Present isolate	<i>R. oryzae</i> [2]	<i>R. oryzae</i> [3]
Colony	Color Brownish-grey to blackish-grey	Brownish-grey to blackish-grey	Brownish-grey to blackish-grey
Sporangium	Shape Globose	Spherical to hemispheric	Globose
	Size 60~200 $\mu\text{m}$	96~225 $\mu\text{m}$	30~210 $\mu\text{m}$ in diameter
Sporangiospores	Shape Sub-globose or oval	Irregular or oval mostly	Sub-globose, rhomboidal or limoniform
	Size 6~10 $\mu\text{m}$ in length	7~10 $\mu\text{m}$	4~10 $\mu\text{m}$ in length
Sporangiophores	Size 10~20 $\mu\text{m}$ in diameter	12~20 $\mu\text{m}$ in diameter	7~20 $\mu\text{m}$ in diameter
Columella	Shape Globose to sub-globose	-	Globose to sub-globose
	Size 80-110 $\mu\text{m}$ in diameter	-	90 $\times$ 120 $\mu\text{m}$ in diameter

**Fig. 2.** Morphological characteristics of *Rhizopus oryzae* isolated from soft rot lesion on oriental melon (*Cucumis melo*). A, Colony formed on potato dextrose agar 6 days after inoculation; B, Sporangiospores (scale bar = 10  $\mu\text{m}$ ); C, Sporangium and sporangiophore; D, Columella; E, Rhizoids.

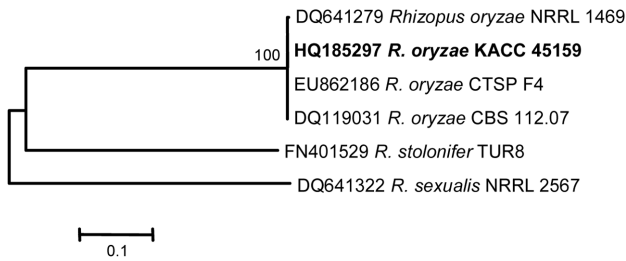
tive humidity at 25°C for 24 hr. Then, the inoculated fruits were placed on a laboratory table at room temperature. The typical symptoms appeared 4 days after inoculation (Fig. 1C and 1D). The infection usually started from the wounded sites of the fruits. The symptoms were identical with those of the naturally occurring disease. Morphological characteristics of the re-isolated fungus from the inoculated fruits were the same as those from the original isolate. Soft rot on Oriental melon caused by *R. oryzae* has not been previously reported in Korea [4]. The representative culture of the fungus has been deposited with the Korean Agricultural Culture Collection (KACC 45159), National Academy of Agricultural Science, Rural Development Administration, Suwon, Korea.

#### Internal transcribed spacer (ITS) sequence analysis.

To identify the causal fungus, the amplification and sequencing of ITS rDNA of the isolate was conducted

using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), as previously described [5]. The resulting 627 bp sequence was deposited in GenBank (accession No. HQ185297). Phylogenetic analysis was performed using MEGA4 with the neighbor-joining method and Tajima-Nei distance model. Previously published ITS sequences from *R. oryzae* strains were included for reference, and *R. sexualis* (GenBank accession No. DQ641322) was used as an out-group. As revealed by the neighbour-joining analysis shown in Fig. 3, the two species had similar ITS sequences. In the phylogenetic tree, the present isolate was placed within a clade comprising reference isolates of *R. oryzae* (Fig. 3).

On the basis of the mycological characteristics, molecular data and pathogenicity to the host plant, the fungus was identified as *R. oryzae*. To our knowledge, this is the first report of the presence of *R. oryzae* on oriental melons in Korea. This occurrence may be an indication that



**Fig. 3.** Phylogenetic tree using internal transcribed spacer sequences showing closest known relatives of *Rhizopus oryzae* including stem rot fungus infecting Oriental melon. 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 the bootstrap values. Bars indicate number of nucleotide substitutions per site. The present isolate infecting Oriental melon was marked in bold.

*R. oryzae* is spreading widely. If so, it may pose a serious threat to these fruits in Korea.

## Acknowledgements

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