

## RESEARCH NOTE

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# Soft Rot of *Rhizopus oryzae* as a Postharvest Pathogen of Banana Fruit in Korea

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Soft rot on banana fruit caused by *Rhizopus oryzae* was identified for the first time in Korea. Colonies were white to light brown and formed numerous sporangiospores. Optimum temperature for mycelial growth was 30°C. Sporangia were globose and 30~200 µm. Sporangioophores were usually straight, 8~20 µm, and rhizoids usually in groups of 3~5. Columella were globose to sub-globose and 90~110 µm. Sporangiospores were sub-globose or oval and 4~10 µm. Based on its mycological characteristics, molecular analysis, and pathogenicity to host plants, this fungus was identified as *Rhizopus oryzae* Went & Prisen Geerlgs. This is the first report of soft rot on banana caused by *Rhizopus oryzae* in Korea.

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

Postharvest losses as a result of fungal infection occur when products are stored for an extended period of time at cold or high temperatures, or as a result of mechanical damage during storage or transport [1]. In order to prevent postharvest losses caused by fungal infection, products must be sound, clean, whole, fresh, free of abnormal moisture, and undamaged.

A disease suspected to be *Rhizopus* soft rot was observed on banana (*Musa sapientum*) fruit placed on local market shelves in Jinju, South Korea in July 2011 (Fig. 1A). Symptoms on banana fruit were similar to those of soft rot caused by *Rhizopus stolonifer* [2]. Infection usually started from the cracks and occurred at harvest time. The disease was not serious in the preclimacteric stage while still green and hard, but losses were considerable when bananas reached the climacteric stage.

The objective of this study was to isolate and identify the soft rot fungus associated with recent outbreaks of soft rot on banana fruit in South Korea. In this research, we focused on mycological characteristics, molecular analysis, and pathogenicity tests on banana fruit.

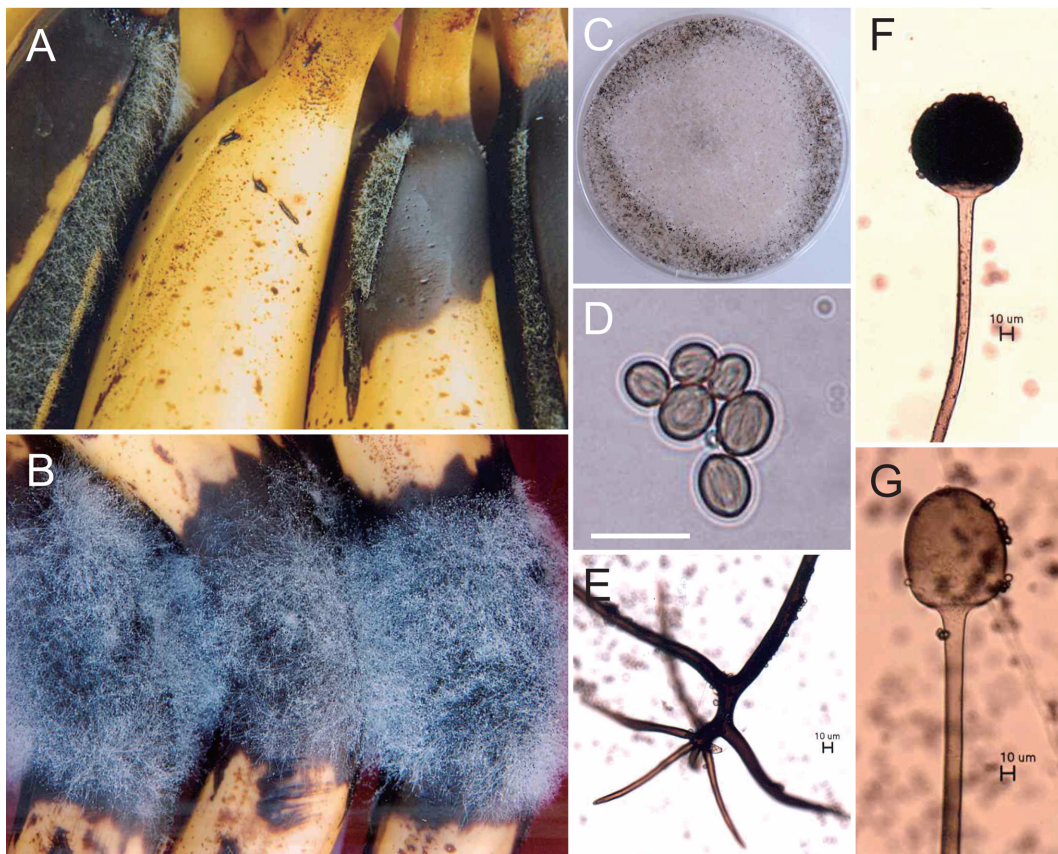
The causal fungus was isolated from diseased banana fruit sampled from a wholesale market. Mycological characteristics of sporangiospores, sporangia, and sporangioophores were observed under a light microscope (Axioplan; Carl Zeiss,

Jena, Germany) (Table 1) [3]. Fungal colonies on potato dextrose agar were white, then became brownish-grey to blackish-grey and spread rapidly with stolons fired at various points to the substrate by rhizoids (Fig. 1C). The optimum temperature for mycelial growth was 30°C, with good growth observed at 37°C. Sporangia were globose, white at first, and then turned black with many spores, mostly 30~200 µm (Fig. 1F). Sporangiospores were unequal, numerous, irregular, sub-globose or oval, angular with striations, and 4~10 µm (Fig. 1D). Rhizoids and stolons were dark brown (Fig. 1E). Sporangioophores 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 (Fig. 1F). Columella were globose to sub-globose, pale brown, and mostly 90~110 µm (Fig. 1G).

For the pathogenicity test, six banana fruits were artificially inoculated with a representative fungus using the wound infection method. For this, a conidial suspension (0.1 mL,  $2 \times 10^4$  conidia/mL) of the causal fungus was placed on the surface of the banana fruits. Inoculated fruits were kept in a moist chamber with 90% relative humidity at 30°C. After 3 days of incubation, the same fungal symptoms were reproduced on inoculated banana fruits (Fig. 1B). The causal pathogen was re-isolated from lesions in order to confirm Koch's postulates.

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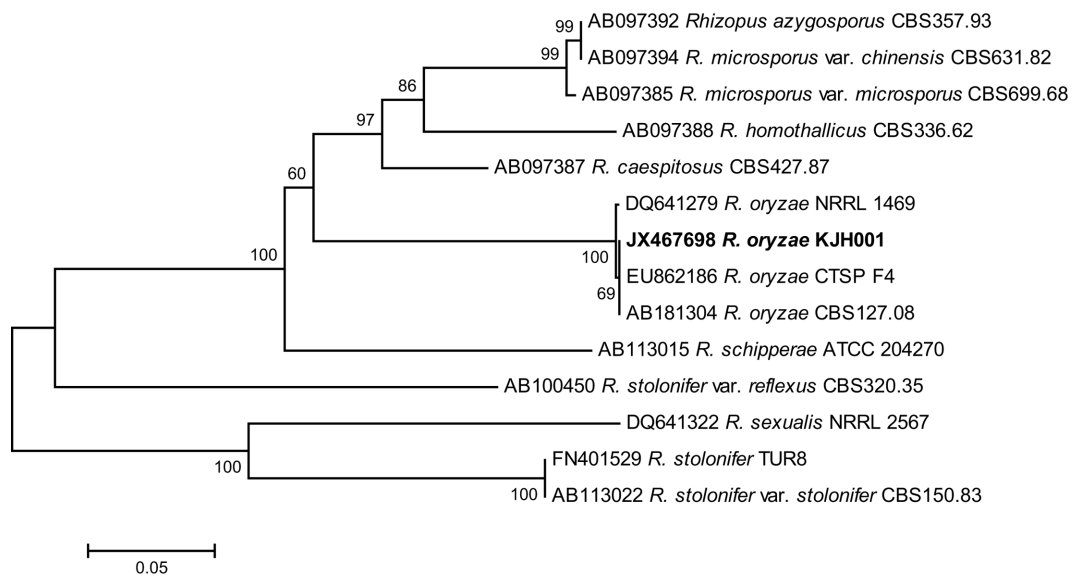
**Fig. 1.** Symptoms and morphological characteristics of soft rot caused by *Rhizopus oryzae* on banana fruit. A, Soft rot symptoms on banana fruit sampled from a local market; B, Symptoms induced by artificial inoculation. C, Colony on potato dextrose agar after 7 days of incubation; D, Sporangiospores (scale bar = 10 µm); E, Rhizoids; F, Sporangium and sporangiophore; G, Columellum.

**Table 1.** Comparison of morphological characteristics of soft rot fungus isolated from banana fruit with previous descriptions of *Rhizopus oryzae*

Characteristics		Isolate in present study	<i>R. oryzae</i> [3]
Colony	Color	Brownish-grey to blackish-grey	Brownish-grey to blackish-grey
Sporangium	Shape	Globose	Globose
	Size (µm)	30~200	30~210
Sporangiospore	Shape	Sub-globose or oval	Sub-globose, limoniform
	Size (µm)	4~10	4~10
	Size (µm)	8~20	7~20
Columellum	Shape	Globose to sub-globose	Globose to sub-globose
	Size (µm)	90~110	90~120

To confirm the identity of the causal fungus, the complete internal transcribed spacer (ITS) rDNA of the isolate was amplified using primers ITS1 (5'-TCCGTAG-GTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTAT-TGATATGC-3') [4]. Total DNA was isolated using an Exgene Plant-Fungal SV mini kit (Geneall Biotechnology Co., Seoul, Korea), following the manufacturer's instructions. PCR reaction contained 5 units of *Taq* polymerase (Takara, Tokyo, Japan), 1 × PCR buffer, 0.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 5 pmol of each primer, and approximately 10 ng of fungal genomic DNA in a total volume of 50 µL

adjusted with sterile water. PCR reaction was performed on an Astec PC 802 thermal cycler (Astec, Fukuoka, Japan) using the following thermal profile: 98°C for 2 min, followed by 30 cycles of 98°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and a final extension step of 72°C for 4 min. Amplified products were electrophoresed through a 0.8% agarose gel in 1 × TBE buffer at 100 V for 20 min. PCR amplicons were extracted by agarose gel electrophoresis using a gel extraction kit (Geneall Biotechnology Co.). Purified PCR products were cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA) in order to generate



**Fig. 2.** Phylogenetic tree using internal transcribed spacer (ITS) sequences showing closest known relatives of *Rhizopus oryzae*, including soft rot fungus infecting banana fruit. 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 banana fruit is marked in bold.

plasmid pOR9. Sequencing was performed using a Bigdye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) using primers M13F and M13R, following the manufacturer's instructions. The resulting 627-bp of the ITS rRNA gene sequence was deposited in GenBank (accession No. JX467698).

Phylogenetic analysis was performed using MEGA4 with the neighbor-joining method and the Tajima-Nei distance model. Previously published ITS sequences from *R. oryzae* strains were included for reference [5]. In the phylogenetic tree, the present isolate was placed within a clade comprising *R. oryzae* reference isolates (Fig. 2).

Soft rot of banana caused by *R. stolonifer* has been reported previously [2], whereas soft rot caused by *R. oryzae* has not been recorded in Korea [6]. Based on its mycological characteristics, molecular data, and pathogenicity to host plants, the fungus was identified as *Rhizopus oryzae* Went & Prisen Geerligs [3]. This is the first report of *R. oryzae* infection on banana in Korea.

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