Mycobiology 34(3): 154-157 (2006)

Characterization of *Sclerotinia sclerotiorum* Isolated from Paprika

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(Received September 15, 2006)

A fungal isolate collected from infected paprika (*Capsicum annuum* var. *grossum*) was characterized as *Sclerotinia sclerotiorum* based on its ability of sclerotium formation, physiological and molecular properties. When the isolate was grown on potato dextrose agar, oatmeal agar, and malt extract agar, it grew most well on PDA. Optimal temperature and pH for its growth were 25°C and pH 7, respectively. The fungal isolate produced sclerotia on PDA within 10 days, and the color and shape of the sclerotia were similar to those of *S. sclerotiorum*. The ITS rDNA regions including ITS1 and ITS2 and 5.8S sequences were amplified using ITS1F and ITS4 primers from the genomic DNAs of the paprika isolate and other known pathogenic *S. sclerotiorum* isolated from different crops in Korea, and their nucleotide sequences were determined. Sequence comparison analysis showed the ITS rDNA of the paprika isolate shares 100% sequence identity with those of *S. sclerotiorum* isolated from red pepper, lettuce and a *S. sclerotiorum* isolate registered in GenBank DNA database. Neighbor joining analysis based on the ITS rDNA sequence revealed the paprika isolate has very close phylogenetic relationships with known *Sclerotinia sclerotiorum* isolates. This is the first report that *S. sclerotiorum* has been found associated with paprika rot in paprika growing countries.

KEYWARDS: ITS rDNA, Paprika, *Sclerotinia sclerotiorum*, Stem rot

Paprika (*Capsicum annuum* var. *grossum*) is a vegetable crop which is principally used as an ingredient in a broad variety of dishes throughout the world. This mild pepper is also referred to as bell pepper or sweet pepper. This foreign origin pepper was first cultivated in Korea in Jeju Island in 1994. Although this vegetable is not popular in Korean food market, its cultivation and production have been continuously increased to export Japanese market as a highly profitable crop. With the increase of its cultivation, recently there have been reports on the occurrence of new diseases in paprika in Korea. These include fusarium rot by *Fusarium solani* (telemorph *Nectria haematococca*) (Ji *et al.*, 2005) and virus diseases such as *Cucumber mosaic virus* (Kim *et al.*, 2002), *Potato virus Y* (Choi *et al.*, 2005), and *Tomato spotted wilt virus* (Kim *et al.*, 2004). In this study we characterized a fungal isolate that was present on the diseased stem of paprika grown in a greenhouse of a paprika farm at Anseong-Si, Gyeonggi-Do in July, 2005.

The fugal isolate was collected from the rotten stem of paprika (Fig. 1A). The leaves and fruits above the diseased stem part were wilted and the diseased lesion was water-soaked with brownish color. White mycelium usually covered diseased stems and dark scleotia were found on the diseased stem of paprika (Fig. 1B). For the isolation of the fungus associated with the rotten paprika stem, the diseased paprika stem was split and several pieces (25 mm^2) of lesion tissues were taken from both inside and outside of the split paprika stem. After surface-sterilization with 5% sodium hypochlorite by soaking for 1 min and rinsing with sterile water three times, and the lesion tissues were placed on PDA plates, incubated for 1~2 days at 25°C. The fungal mycelia grown out from the lesion tissues were transferred to new PDA plates and cultured for a week at 25°C to examine morphological features. Several fungal isolates were obtained from the lesion tissues, but all the isolates were considered an identical species because they shared common colony morphology, showed very similar growth pattern on PDA, and produced scleotia similarly on PDA. The colony on PDA was white, gray and contained small black sclerotia (Fig. 1C). The size of sclerotia were usually about 3~10 mm long × 1~2 mm wide, with a black outside covering and a white interior (Fig. 1D, 1E). The color of scleotia was black, and the shape of scleotia was globose and cylindrical or irregular. The isolate could produce 15 to 20 sclerotia on PDA per a Petri plate. Confrontation of two colonies of the isolate on PDA tended to slightly enhance the speed of sclerotia formation. Based on these colony properties the fungal isolate was considered as a *Sclerotinia* species.

We further examined the growth properties of the *Sclerotinia* isolate by growing it on different culture media and at diverse temperature and pH ranges. When the *Sclerotia* isolate was grown for 6 days at 25°CC on PDA (potato dextrose agar), MEA (malt extract agar), OMA (oatmeal agar), PDA was the best for the mycelial growth of the isolate (Fig. 2B). Colony diameter of the fungal

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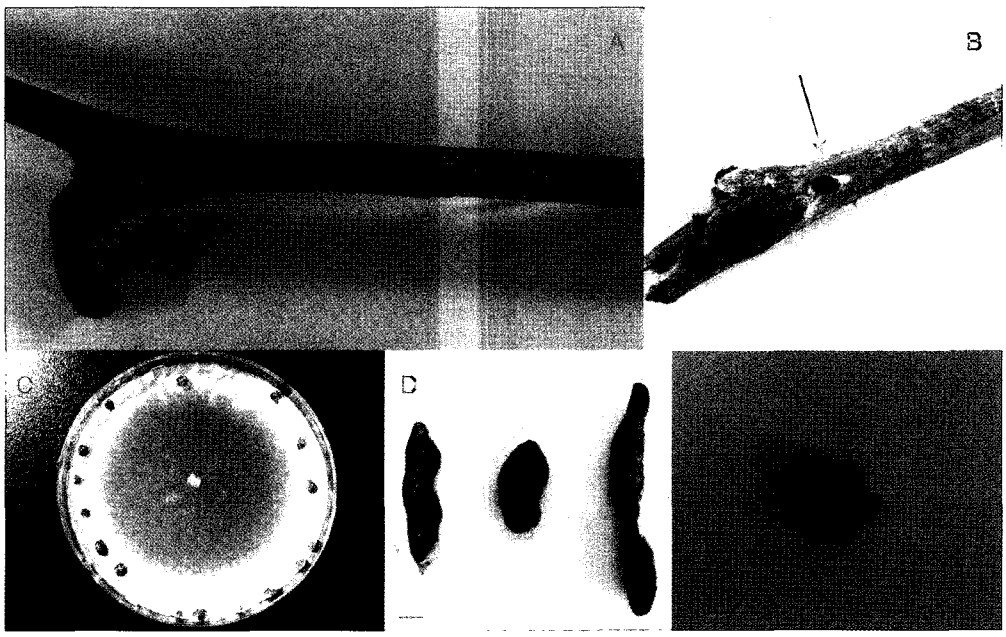

Fig. 1. Paprika plants infected by the *Sclerotinia* isolate in a greenhouse. A: symptom in the stem part of a paprika plant. B: dried-gray colored middle stem at the late stage of infection (the arrow indicates a sclerotium). C: colony and sclerotia of the isolate grown on PDA. D and E: sclerotia in different sizes and dissection (the bar represents 1 mm).

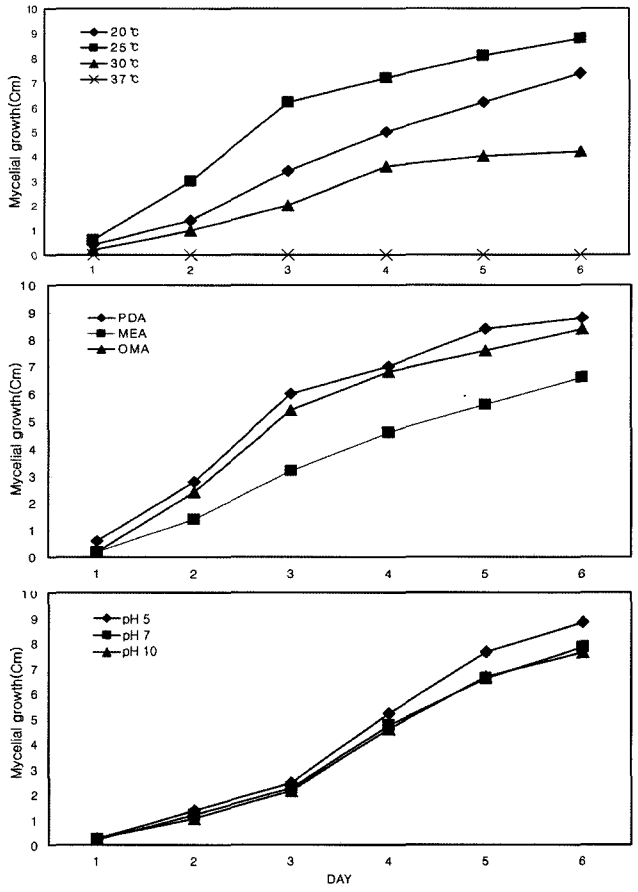

Fig. 2. Growth properties of the *Sclerotinia* isolate from paprika. The isolate was grown at 25°C for 6 days on different temperature (top), media (middle), and pH (bottom).

isolate reached to 44.4 mm on PDA after 7 days at 25°C. OMA was the next with colony diameter of 41.5 mm. The fungal isolate had the slowest growth on MEA. The *Sclerotinia* isolate favored 25°C for its best growth (Fig. 2A). The fungus could not grow at 37°C. In preliminary study the fungal isolate showed its ability of growth at broad rages of pH. Thus narrow pH ranges of 5, 7, 10 were compared to define more precise optimum pH. The highest mycelial growth was shown at pH 7 (Fig. 2C). Overall the growth properties of mycelium in the paprika isolate were generally agreed with those of known *Sclerotinia* fungi.

We tried to induce the formation of apothecia but it was not successful. Therefore, we performed molecular identification. After growing the fungal isolate on cellophane sheet-layered PDA for 5 days, genomic DNA preparation and PCR amplification of the ITS rDNA region including ITS1 and ITS2 and 5.8S sequences using ITS1F and ITS4 primers performed using the method described by Kim *et al.* (1999). PCR amplified approximately 600 bp of the ITS ribosomal DNA region. Amplified PCR product was detected on 1% agarose gel through electrophoresis. Checked amplicon was purified with AccuPrep® PCR Purification Kit (Bioneer Corp., Daejeon, Korea). The Purified PCR product was sequenced with ABI 3730xl sequencer (Applied Biosystems) using ITS1F primer as a sequencing primer (White *et al.*, 1990). Homology search through GenBank DNA database revealed the determined ITS rDNA sequence of the paprika isolate share 100% sequence identity with that of *Sclerotinia sclerotiorum* B52 isolate collected from an unknown vegeta-

ble crop in USA (GenBank accession number DQ329538).

To further confirm the molecular results, we obtained four reference cultures of *S. sclerotiorum* having Korean origin (KACC41065 from red pepper, KACC40457 from lettuce, KACC41069 from potato, and KACC40172 from tangerine) from Korean Agricultural Culture Collection (KACC), sequenced and compared their ITS rDNA. Sequence comparison in the GenBank DNA database displayed the ITS rDNA sequences of the four *S. sclerotiorum* cultures have highest sequence identity with that of *S. sclerotiorum*. These results indicate that all the four Korean KACC cultures are *S. sclerotiorum*. To define their relationship to the paprika isolate, phylogenetic analysis was carried out based on the ITS rDNA data set using PAUP*4.0b10 (Swofford, 2001). For phylogenetic analysis, other species of *Sclerotinia* was used as an outgroup and a suboutgroup, respectively. Sequences generated in this study were aligned with those retrieved from GenBank using CLUSTAL W (Thompson *et al.*, 1994), ambiguous and uninformative variable sites were excluded, and gaps were treated as missing data. Neighbor-joining tree was constructed using Kimura's two parameter model. Tree topology was tested with bootstrap analysis with 1000 replication. The paprika isolate in this study closely grouped with *S. scleotiorum* isolates (Fig. 3). Within *S. scleotiorum* isolates, the paprika isolate revealed more close phylogenetic relationship with isolates from red pepper, lettuce and vegetable crop than tangerine and potato hosts. All the *S. scleotiorum* isolates were separately related with other *Sclerotinia* species. This phylogenetic data again strongly support that the paprika isolate is *S. scleotiorum*. It remains if the source of inoculm of *S. scleotiorum* in paprika greenhouses is domestic origin or foreign countries, especially from Holland.

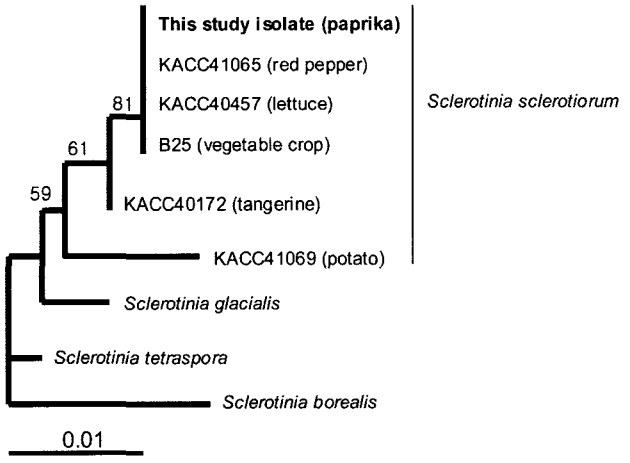

Fig. 3. Phylogenetic relationship of the *Sclerotinia* isolate from paprika to other *Sclerotinia sclerotiorum* isolates and *Sclerotinia* species. Phylogenetic tree was recovered from the neighbor-joining analysis of the nuclear ITS rDNA sequence data. Numbers above nodes are bootstrap intervals. *Sclerotinia borealis* was used as an outgroup taxon.

Sclerotinia sclerotiorum appears to be among the most nonspecific, omnivorous, and successful of plant pathogens (Abams and Ayers, 1979; Kohn, 1979). Purdy (1979) has identified a host range within taxonomic groups as follows: 64 plant families, 225 genera, 361 species, and 22 other (cultivars, etc.), for a total of 383 species and other categories. In Korea, more than 30 species of its host plants were reported. On the basis of mycological characteristics and molecular analysis, the fungal isolate from paprika was characterized to *Sclerotinia sclerotiorum*. The present study revealed that *S. sclerotiorum* is associated with sclerotinia rot in paprika in Korea. To our knowledge, this is the first report of the occurrence of sclerotinia rot on paprika in Korea as well as in other paprika cultivation countries.

Acknowledgement

This study was carried out with the support of "Specific Joint Agricultural Research-promoting Projects (Project No. 20060101033237)", RDA, Republic of Korea.

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