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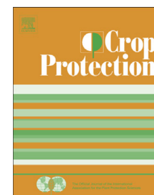

Short communication

Penicillium brasilianum as a novel pathogen of onion (*Allium cepa* L.) and other fungi predominant on market onion in Korea

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ABSTRACT

Onion (*Allium cepa* L.) is an important vegetable crop in Korea, but its production is severely affected by fungal pathogens during plant growth and bulb storage. We investigated the occurrence of fungi on market onion bulbs; identified the predominant fungal species based on the internal transcribed spacer region, β-tubulin region, and elongation factor 1-α gene sequences; and tested the pathogenicity of each predominant fungal species in onion bulbs. The genera *Aspergillus* (63.9%), *Penicillium* (15.5%), *Fusarium* (6.4%), *Rhizopus* (5.2%), and others (9.0%) were detected in the samples. Among these genera, *Aspergillus awamori*, *Fusarium oxysporum*, *Penicillium brasilianum*, and *Rhizopus oryzae* were identified as the predominant species. All of the fungi tested could infect both the inner layers and outer surfaces of onion bulbs and be re-isolated from the infected tissues. To our knowledge, this is the first report that *P. brasilianum* is a fungal pathogen of onion bulbs.

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Onion (*Allium cepa* L.) is widely grown as a source of food worldwide (McDonald et al., 2004), and it is an important vegetable crop in Korea occupying a total cultivation area of 20,957 ha in 2012 (Statistics Korea; www.kostat.go.kr). Onions are propagated from seeds and are planted directly or transplanted into fields. After harvest, onion bulbs are normally placed in storage houses (McDonald et al., 2004). In Korea, onions are planted in the autumn and harvested in the following April to June. Therefore, long-term storage of onion bulbs is necessary to ensure a steady supply throughout the year. The harvest in Korea usually occurs during the rainy season, and occasionally moisture is insufficiently removed from bulbs before long-term storage (Cho et al., 2010). Consequently, physiological changes can take place in onion bulbs including enhanced respiration and the production of ethylene and other compounds during long-term storage (Cho et al., 2010; Song et al., 2009). These conditions may promote the growth of postharvest plant pathogens, some of which initially establish themselves in early phases of plant growth (Gent and Schwartz, 2005; McDonald et al., 2004). In addition, certain plant pathogens can be transmitted from diseased seeds or seedlings that have been grown in the field to postharvest bulbs (Özer and Köycü, 2004).

To control onion diseases, various management strategies have been adopted, *e.g.*, maintaining well-drained soil, controlling nitrogen levels, soil solarization and chemical applications, use of disease-free planting materials, reducing wounding during harvest and storage, and curing (Carisse et al., 2011; Gent and Schwartz, 2005; McDonald et al., 2004). In particular, disease management during the postharvest stages in storage houses is important to maintain the quality of marketable onion bulbs. As an initial step toward this goal, information regarding the occurrence of major fungal pathogens in onion bulbs is critical as observed in other stored products (Oh et al., 2007, 2010). Several fungal pathogens of onion bulbs in Korea, such as *Fusarium oxysporum*, *Aspergillus* sp., and *Botrytis allii*, have previously been reported (Lee et al., 2001). Further information on fungal postharvest pathogens of onion in Korea is neither recent nor comprehensive. Thus, we (i) investigated the occurrence of fungi in onion bulbs from a commercial market, (ii) identified each predominant fungal species from the onion bulbs by analysis of the internal transcribed spacer (ITS) region, β-tubulin region, or elongation factor 1-α gene sequence, and (iii) tested the pathogenicity of each predominant fungal species against onion bulbs.

Diseased onion bulbs originating from Muan, Jeonnam province were obtained three times [3 (96), 9 (52), and 12 (45 bulbs) August 2013] from the Garak Market, which is the largest commercial

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market in Seoul, Korea. Each onion bulb showed one to two apparent lesions. For fungal isolation from the diseased onions, lesion pieces (four pieces per lesion) from each bulb (total of 193 bulbs) were sterilized with 1% NaOCl for 1 min, washed three times with sterile distilled water, and then blotted on a sterile filter paper (Whatman No. 1). These pieces were placed on acidified potato dextrose agar (PDA, pH 3.9) supplemented with lactic acid (600 μl L^{-1}) at 28 °C and observed daily for 7 d. The mycelia grown from the pieces (mainly one isolate per lesion) were transferred to acidified PDA and further cultured at the same temperature. Using these fungal cultures, hyphal-tip cultures were conducted for further experiments.

Fungal isolates that were obtained from the onion bulb samples were grouped on the basis of their distinct mycelial and/or conidial morphologies. Isolates with black colonies and delicate roughened conidia were classified as the genus *Aspergillus*; isolates with blue green conidia and velutinous surface structure were classified as the genus *Penicillium*; isolates with white floccose mycelium and pink to purple reverse color were classified as the genus *Fusarium*; and isolates with white floccose mycelium and black conidial head were classified as the genus *Rhizopus*. Thereafter, a representative isolate (GR-105, GR-126, GR-137, or GR-172) from each fungal group (*Penicillium*, *Fusarium*, *Aspergillus*, or *Rhizopus*) was selected and subjected to cultural, morphological, and molecular identification. For cultural and morphological observations, isolate GR-137 was subjected to observation of colony color and texture 7 d after growth on Czapek yeast extract agar at 25 °C (CYA25) and 37 °C (CYA37) and malt extract agar (MEA) (Klich, 2002), and isolate GR-105 was observed 7 d after growth on CYA25, CYA37, MEA, Czapek yeast autolysate with 5% NaCl (CYAS), dichloran 18% glycerol agar (DG18), yeast extract sucrose agar (YES), and creatine sucrose agar (CREA) (Oh et al., 2007). In addition, isolate GR-126 was grown on Spezieller Nährstoffarmer agar (SNA) for macro- and micro-conidium formation (Leslie and Summerell, 2006), and isolate GR-172 was grown on PDA for sporangium and spore observations.

For molecular identification of the representative isolates, the genomic DNA was extracted from fungal mycelia grown on PDA at 28 °C for 7 d using an i-genomic BYF DNA Extraction Kit (iNtRON Biotechnology, Sungnam, Korea) according to the manufacturer's instructions. The ITS regions of all selected isolates were amplified using the universal primers ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) (Glass and Donaldson, 1995). Furthermore, the β-tubulin region of isolates GR-137 and GR-105 was amplified using primers Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Glass and Donaldson, 1995). The elongation factor 1-α gene of isolate GR-126 was amplified with primers EF-1 (5′-ATGGGTAAGGAAGACAAGAC-3′) and EF-2 (5′-GGAAGTACCAGTGATCATGTT-3′) (Kim et al., 2005). Polymerase chain reaction (PCR) and DNA sequence and phylogenetic analyses were conducted as described by Kim et al. (2009) and Sang et al. (2013). The experiments were conducted twice and they produced similar results.

For pathogenicity tests of the selected fungal isolates on onion bulbs, isolates GR-105, GR-126, GR-137, and GR-172 were grown on PDA at 28 °C for 5 d. Fungal mycelia (diameter of 5 mm) from the edges of the growing cultures or PDA plugs (inoculated control) were inoculated on the inner layers of the half-cut onion bulbs and the outer surfaces of the bulbs (four points [sub-replicates] per bulb [replicate]) including an inoculated control (one point), in which the outer surfaces were slightly scratched using sterile sandpaper. However, isolate GR-172 was inoculated at a point on the inner layers and the outer surfaces of the onion bulbs because it produced large lesions. Slight scratches on the outer surfaces of the bulbs were used as uninoculated controls. The inoculated onion bulbs were then placed on mesh screens in containers (16.0 × 23.0 × 12.5 cm) with two layers of wet paper towels and incubated at 28 °C for 5 d. The lesion diameter (mm) was measured 5 d after inoculation (DAI) for all tested isolates on the inoculated bulbs, except for isolate GR-172 (3 DAI). This experiment was conducted twice with three replicates.

Data were analyzed using Statistical Analysis Systems software (SAS Institute, Cary, NC, USA). Data from repeated experiments were pooled after the homogeneity of the variances was confirmed using Levene's test (Levene, 1960) and further statistically analyzed. General linear model procedures were used for the analysis of variance, and the means were separated using the least significant difference test at $P < 0.05$.

In total, 233 fungal isolates were obtained from three trials for isolation of fungi from the lesions of the onion bulbs sampled from a commercial market (Table 1). Among the isolates, 212 isolates from the market onion bulbs belonged to four fungal genera *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus*. Among these genera, *Aspergillus* showed the highest frequency of occurrence (63.9%), followed by *Penicillium* (15.5%), *Fusarium* (6.4%), *Rhizopus* (5.2%), and others (9.0%) (Table 1).

Among the genera *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus*, each representative isolate (*Aspergillus* GR-137, *Fusarium* GR-126, *Penicillium* GR-105, or *Rhizopus* GR-172) (Fig. 1) was selected for cultural, morphological, and phylogenetic analyses. In cultural and morphological observations, the conidia of *Aspergillus* GR-137 on CYA25 were almost black, and the mycelium was white with the reverse side dull brown, light brown, or grey brown. The colonies were low, granular, and radially sulcate with a diameter of 54.9 mm. On CYA37, colonies were similar to those of CYA25 except that the mycelium was inconspicuous and the reverse side was dull brown to dark brown. On MEA, the mycelium was inconspicuous to white, and colonies were dark brown and planar with an uncrowded conidial head.

Colonies of *Penicillium* GR-105 on CYA25 had a diameter of 26.0 mm and were slightly radially sulcate, velutinous, or somewhat floccose. The mycelium was white and conidiogenesis was heavy with greyish green conidia. Soluble pigment was absent, and the reverse side was yellow to yellowish brown. On CYA37, colonies were irregularly sulcate and centrally depressed. The mycelium was white and conidiogenesis was light with blue green or grey green conidia. Soluble pigment was absent, and the reverse side was dark yellow to reddish yellow. On MEA, colonies were planar and velutinous. The mycelium was white and conidiogenesis was moderate to heavy with blue green conidia. Soluble pigment was absent, and the reverse side was pale yellow. On CYAS, colonies were sulcate, centrally depressed, and velutinous. The mycelium was white to inconspicuous and conidiogenesis was heavy with blue green to greyish green conidia. Soluble pigment was absent, and the reverse side was yellow to yellowish brown. On DG18, colonies were planar, centrally floccose, and velutinous to somewhat floccose. The mycelium was white to inconspicuous and conidiogenesis was heavy with blue green conidia. Soluble pigment was absent, and

Table 1
Fungal genera isolated from onion bulbs from the Garak commercial market, Seoul, Korea in 2013.

Fungal genera	Isolate (number)				Frequency of occurrence (%)
	Trial 1	Trial 2	Trial 3	Total	
Aspergillus	56	48	45	149	63.9
Fusarium	2	5	8	15	6.4
Penicillium	10	16	10	36	15.5
Rhizopus	5	1	6	12	5.2
Others	8	7	6	21	9.0
Total	**81**	**77**	**75**	**233**	**100**

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