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Isolation and Identification of Fungal Species from the Insect Pest *Tribolium castaneum* in Rice Processing Complexes in Korea

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The red flour beetle, *Tribolium castaneum*, is one of the most common and economically important pests of stored cereal products worldwide. Furthermore, these beetles can act as vectors for several fungal post-harvest diseases. In this study, we collected *T. castaneum* from 49 rice processing complexes (RPCs) nationwide during 2016-2017 and identified contaminating fungal species on the surface of the beetles. Five beetles from each region were placed on potato dextrose agar media or *Fusarium* selection media after wet processing with 100% relative humidity at 27°C for one week. A total of 142 fungal isolates were thus collected. By sequence analysis of the internal transcribed spacer region, 23 fungal genera including one unidentified taxon were found to be associated with *T. castaneum*. The genus *Aspergillus* spp. (28.9%) was the most frequently present, followed by *Cladosporium* spp. (12.0%), *Hyphopichia burtonii* (9.2%), *Penicillium* spp. (8.5%), *Mucor* spp. (6.3%), *Rhizopus* spp. (5.6%), *Cephaliophora* spp. (3.5%), *Alternaria alternata* (2.8%) and *Monascus* sp. (2.8%). Less commonly identified were genera *Fusarium*, *Nigrospora*, *Beauveria*, *Chaetomium*, *Coprinellus*, *Irpex*, *Lichtheimia*, *Trichoderma*, *Byssochlamys*, *Cochliobolus*, *Cunninghamella*, *Mortierella*, *Polyporales*, *Rhizomucor* and *Talaromyces*. Among the isolates, two known mycotoxin-producing fungi, *Aspergillus flavus* and *Fusarium* spp. were also identified. This result is consistent with previous studies that surveyed fungal and mycotoxin contamination in rice from RPCs. Our study indicates that the storage pest, *T. castaneum*, would play an important role in spreading fungal contaminants and consequently increasing mycotoxin contamination in stored rice.

Keywords **:** fungi, mycotoxin, rice processing complexes, *Tribolium castaneum*

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The control of pests in stored grains is as economically important as increasing the crop yield because, unlike crop damage during the growing season, post-harvest damage of stored grains is not financially compensated. Fungi and animal pests are the major culprits for damage of stored grains, globally estimated to be responsible for 20% of food losses and up to 40-50% in some developing countries.

The red flour beetle (*Tribolium castaneum* Herbst) is one of the most important pests for stored grains such as rice (Kim and Ryoo, 1982), maize (LeCato and Flaherty, 1973), millet (Roorda et al., 1982), sorghum (Shazali and Smith, 1986), and wheat flour (Birch, 1945; Daniels, 1956) worldwide. Furthermore, *T. castaneum* beetles cause additional damage by spreading and promoting fungal contamination (Karunakaran et al., 2004; Kim and Ryoo, 1982; Simpanya et al., 2001). Here we chose to investigate the fungal contaminants disseminated by *T. castaneum*.

The *T. castaneum* has been reported to increase the moisture and temperature of stored grains to create an environment favorable for fungal proliferation, thereby accelerating grain degradation and decay (Miller, 1995). Degradation of stored grain by fungi results in lower germination rate, weight loss, loss of nutrients, odor and discoloration, which reduce overall grain quality. Fungal contamination of stored grains not only results in enormous economic losses but also has harmful consequences on human health and livestock due to toxic fungal secondary metabolites called mycotoxins (Tipples, 1995).

T. castaneum has also been reported to act as a vector for these toxigenic fungi during storage (Philip and Throne, 2010). When maize flour is co-contaminated with *T. castaneum*, toxigenic fungi including *Aspergillus* spp. are approximately 5 times more abundant than in the absence of the beetle vectors (Simpanya et al., 2001). Unlike in other stored grains such as wheat, barley, and corn, insect-mediated fungal toxin contamination in stored rice has yet to be reported (Tanaka et al., 2004).

Aspergillus spp., which produces aflatoxin in contaminated rice, has been reported to occur mainly in high temperature and high humidity countries such as India, China, and Iran (Rahmania et al., 2011; Reddy et al., 2008). It has been reported that deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and fumonisin (FMS), which are mycotoxins of genus *Fusarium*, were detected in stored rice (Abbas et al., 1998; Lee et al., 2011; Tanaka et al., 2004). Previously, the distribution of toxigenic fungi on rice was investigated in the southern and central regions of Korea and ochratoxin A (OTA), aflatoxin B_1 (AFB_1), fumonisin B_1 (FB_1), and zearalenone (ZEN) were detected (Park et al., 2005). According to other reports, which tested the geographic distribution of toxigenic fungi contaminating seven different types of rice samples (paddy, husk, brown, blue-tinged, discolored, Broken and polished)from rice processing complexes, *Fusarium* spp. and *Alternaria* spp. were common in the southern region, while *Aspergillus* spp. and *Penicillium* spp. were common in the central region of Korea (Son et al., 2011).

Most studies previously conducted in Korea have focused on the regional distribution of contaminating fungal species, and only a few studies have examined the effect of *T. castaneum* on fungal transmission (Kim and Ryoo, 1982). The purpose of this study was to investigate what type of fungi could be disseminated by *T. castaneum* collected at rice processing complexes by time periods. This study could be used as a reference for establishing a system to effectively protect stored agricultural products.

Materials and Methods

Study site and insect trapping. *T. castaneum* beetles were collected in 49 different rice processing complexes (RPCs) nationwide, over three collection dates between April 2016 and August 2017 (Fig. 1A). We installed three or four corrugated traps (300 × 300 × 2 mm) at each RPC. Traps were placed in a variety of positions on the grain surface and collected a week later, placed individually into plastic bags. In the laboratory, the *T. castaneum* adults from the trap were placed into an insect breeding box (72 × 72 × 100 mm) and stored until just before the experiment.

Isolation of fungi from *T. castaneum* adult. The boxes containing *T. castaneum* were transferred to -15°C for 30 min (Fields, 2012), then five *T. castaneum* individuals were each placed on a sterilized glass slide inside a 9 cm Petri-dish lined with a single-layer of wet filter paper. The plates were incubated at 27°C for 7 days. After wet processing, the beetles were transferred onto potato dextrose agar (PDA) media containing streptomycin (50 mg/L) or *Fusarium* selective media (Nash and Snyder, 1962), and incubated at 25°C for 3 to 7 days. The fungal isolates were transferred to PDA medium and identified according to microscopic observations following the taxonomic keys for each genus (Barnett and Hunter, 1972; Samson et al., 1995). All fungal isolates were deposited at the Center for Fungal Genetic Resources (CFGR) at Seoul National University, Seoul, Korea.

Isolation of genomic DNA from fungal cultures. For molecular identification, fungal genomic DNA was extracted from mycelia using DNeasy Plant Mini Kit according to the manufacturer's protocol (Qiagen, Valencia, CA, USA). Using the purified DNA from the collected isolates, the internal transcribed spacer with 5.8 s rDNA was amplified using ITS5/ITS4 (White et al., 1990). For further identification, beta-tubulin, calmodulin, translation elongation factor1 and glyceraldehyde-3-phosphate dehydrogenase sequence data were amplified using primer pairs BT2A/BT2B (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997), CL1/CL2A (O'Donnell et al., 2000), 728F/1569R or 728F/EF2 (Carbone and Kohn, 1999; O'Donnell and Cigelnik, 1997), and GDF1/GDR1 (Guerber et al., 2003), respectively.

PCR reactions were performed using AccuPower PCR Premix (Bioneer, Korea) with an initial denaturation for 5 min at 94°C, 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C, followed by a final extension for 5 min at 72°C. PCR products were confirmed by gel electrophoresis, purified with AccuPower PCR purification kit (Bineer, Korea) and bi-directionally sequenced on both strands with the same primers used for PCR amplification. Sequence assembly was performed using SeqMan program of DNA star (Madison, WI). The obtained nucleotide sequences were used for BLASTn search in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

In the first round of collection (June 4, 2016), 44 fungal strains were obtained from 17 RPCs (Fig. 1B), 46 were collected in the second round from 22 RPCs (May 18, 2017) (Fig. 1C), and 52 in the third round from 26 RPCs (Aug 1, 2017) (Fig. 1D). Based on the NCBI BLAST search results of the ITS sequences and morphological analysis, *Aspergillus* spp. including *A. flavus* were dominant in whole collected periods (Fig. 1E-G).

A total 142 fungal isolates corresponding to 49 species, belonging to 23 genera, were identified from 40 RPCs (Fig. 2A, Table 1 and Table 2). The major fungal species isolated in each sampling period were *Aspergillus* spp. including *A. flavus* (40.9%), *Cladosporium* sp. (15.9%), and *Mucor* spp. (9.1%) in the first round (June 2016) (Fig. 1E), *Aspergillus* spp. including *A. flavus* (28.3%), *Cladosporium* sp. (17.4%), and *Penicillium* spp. (10.9%) in the second round (May 2017) (Fig. 1F), and *Aspergillus* spp. including *A. flavus* (19.2%), *Hyphopichia* sp. (15.4%), Mucor spp. (7.7%), and *Penicillium* spp. (7.7%) for the third round (August 2017).

The major fungal species in whole periods were *Aspergillus* spp. including *A. flavus* (28.2%), *Cladosporium* spp. (12.0%), *Hyphopichia burtonii* (9.2%), *Penicillium* spp. (8.5%), *Mucor* spp. (6.3%), *Rhizopus* spp. (6.3%), *Cephaliophora tropica* (3.5%), *Alternaria alternata* (3.5%), and *Monascus* sp. (2.8%) (Fig. 2B). Less commonly identified

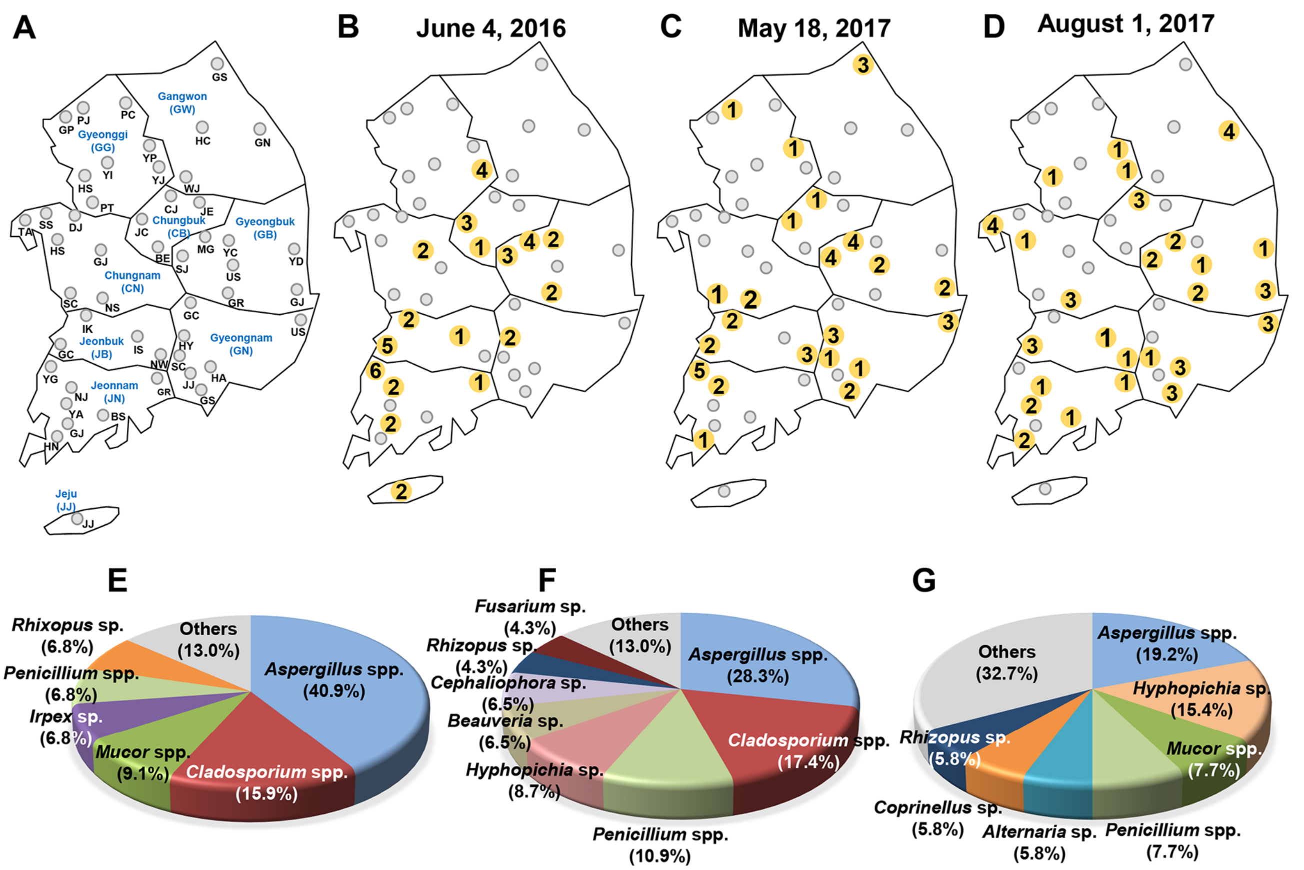

Fig. 1. Collation map of fungal isolates from the insect pest *Tribolium castaneum* in rice processing complexes (RPCs) during 2016 to 2017 in Korea. (A) Distribution map of 49 RPCs in Korea. The 49 RPCs are indicated by gray circles. The name of RPCs are noted by two capitalized letter just below the gray circles. The blue-colored letter indicates 8 provinces in Korea and two capitalized letters in parentheses indicates the abbreviation of provinces (See Table 1). The obtained fungal isolates and distribution map from (B) the first round (44 isolates from 17 RPCs), (C) the second round (46 isolates from 22 RPCs), and (D) the third round (52 isolates from 26 RPCs) of collection. The yellow colored circles indicates the location that obtained fungal isolates from the collected *T. castaneum*. A total number of fungal isolates is noted in the yellow colored circles. The percentage distribution of different fungal isolates from (E) the first round, (F) the second round, and (G) the third round of collection.

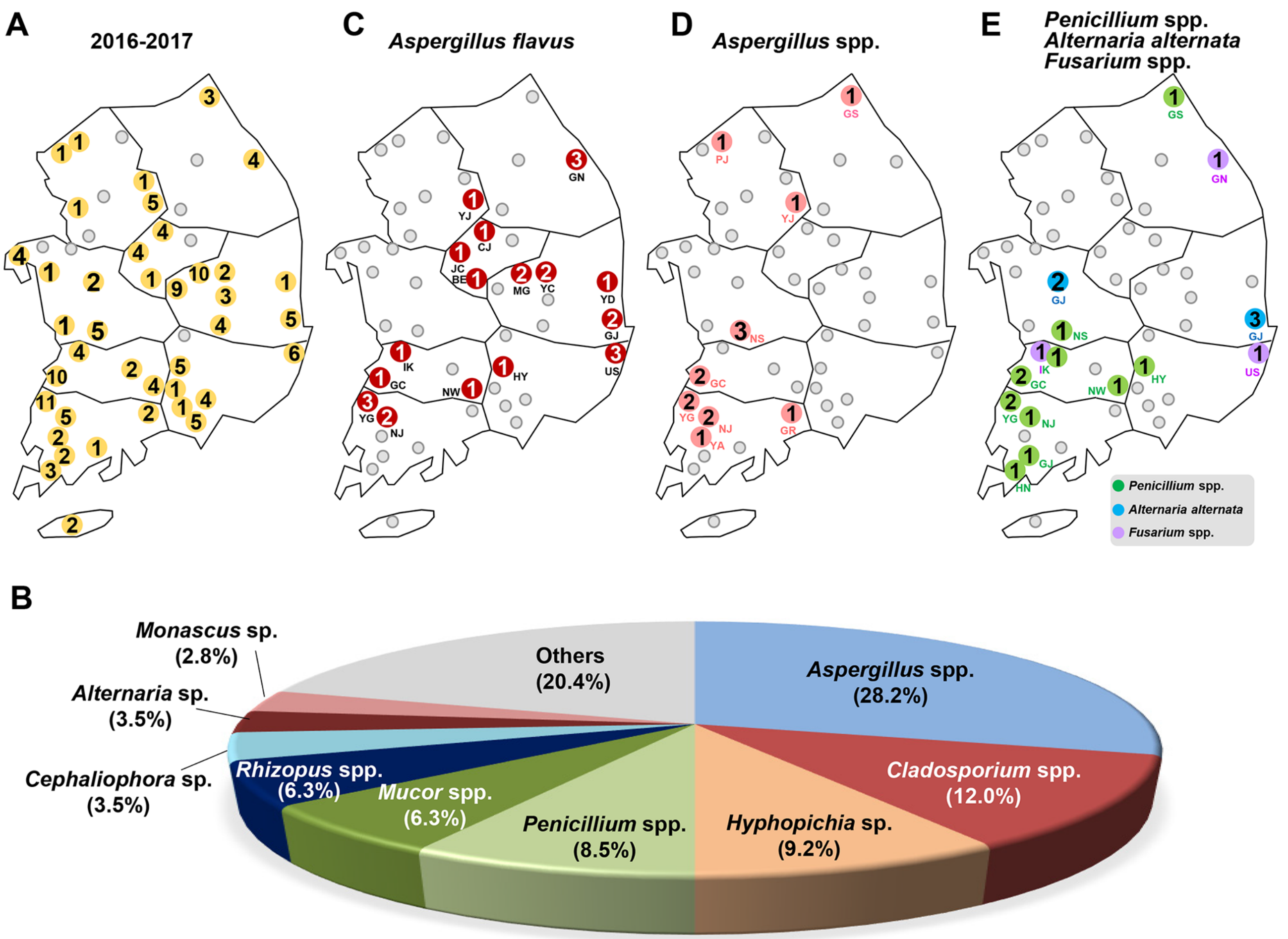

Fig. 2. Summary of fungal isolates from *Tribolium castaneum*. (A) Distribution map of 142 fungal isolates were identified from 40 RPCs. (B) The percentage distribution of different fungal isolates among the collected 142 isolates between April 2016 and August 2017. Distribution map of (C) *Aspergillus flavus* (collected from 12 RPCs), (D) *Aspergillus* spp. (16 RPCs) (E) *Penicillum* spp. (9 RPCs), *Alternaria alternate* and *Fusarium* spp. were collected from *T. castaneum* in this study. The number in the circles indicates a total number of identified isolates.

were *Fusarium* spp., *Beauveria bassiana*, *Chaetomium globosum*, *Coprinellus* sp., *Irpex lacteus*, *Lichtheimia* spp., *Trichoderma* spp., *Byssochlamys spectabilis*, *Cochliobolus miyabeanus*, *Cunninghamella echinulata*, *Nigrospora oryzae*, *Mortierella oligospora*, *Polyporales* sp., *Rhizomucor pusillus*, *Talaromyces* sp. and unidentified fungus. Among the isolates, two known mycotoxin-producing fungi, *Aspergillus flavus* (26 isolates) and *Fusarium* spp. (3 isolates) were identified.

The above results are consistent with a study which demonstrated that *Aspergillus flavus* is the major fungal contaminants of stored wheat in the presence of *T. castaneum* (Bosly and El-Banna, 2015). It is also consistent with another study on stored maize, where 10 species of fungi, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus* sp., *Cladosporium sphaerospermum*, *Fusarium* sp., *Fusarium oxysporum*, *Penicillium* sp., *Mucor* sp., *Mucor racemosus* and *Rhizopus oryzae* were isolated in the presence of *T. castaneum* (Simpanya et al., 2001).

In this study, we found the producer of aflatoxin, *Aspergillus flavus*, associated with *T. castaneum*, collected from16 RPCs (Gangneung (GN) in Gangwon (GW) province, Yeoju (YJ) in Gyeongii, Jincheon (JC), Chungju (CJ), and Boeun (BE) in Chungbuk (CB), Yeongdeok (YD), Gyeongju (GJ), Yecheon (YC) and Mungyeong (MG) in Gyeongbuk, Ulsan (US) and Hamyang (HY) in Gyeongnam (GN), Iksan (IK), Gochang (GC) and Namwon (NW) in Jeonbuk (JB), Yeonggwang (YG) and Naju (NJ) in Jeonnam) (Fig. 2C).

Other *Aspergillus* species were also found on beetles from 9 RPCs (Goseong (GS) in Gangwon province, Paju (PJ) and YJ in Gyeongii, Nonsan (NS) in Chungnam, GC

Table 1. Fungal isolates from *Tribolium castaneum*, GenBank accessions nos. of the ITS region sequences and the Blast search results of the sequences obtained

Isolates	Collection Date	Region, Province	GenBank Accession No.	Most closely related fungi (GenBank Accession No.)	Similarity (%)
CB-BE-3-1	June 4, 2016	Boeun, Chungbuk	MG554270	*Aspergillus* sp. (KX148624.1)	100
CB-JC-3-2	June 4, 2016	Jincheon, Chungbuk	MG554234	*Aspergillus flavus* (AM745114.1)	100
CB-JC-3-3	June 4, 2016	Jincheon, Chungbuk	MG554235	*Mucor circinelloides* (KT336541.1)	99
CB-JC-3-1	June 4, 2016	Jincheon, Chungbuk	MG554233	*Mucor racemosus* (LN809049.1)	99
CN-GJ-3-2	June 4, 2016	Gongju, Chungnam	MG554260	*Alternaria alternata* (HQ380767.1)	100
CN-GJ-3-1	June 4, 2016	Gongju, Chungnam	MG554259	*Alternaria alternata* (HQ380767.1)	100
GB-GR-3-1	June 4, 2016	Goryeong, Gyeongbuk	MG554250	*Irpex lacteus* (KU761586.1)	99
GB-GR-3-2	June 4, 2016	Goryeong, Gyeongbuk	MG554251	*Mucor racemosus* (HM641690.1)	99
GB-MG-3-3	June 4, 2016	Mungyeong, Gyeongbuk	MG554264	*Aspergillus* sp. (KC178674.1)	100
GB-MG-3-4	June 4, 2016	Mungyeong, Gyeongbuk	MG554265	*Aspergillus* sp. (KC178674.1)	100
GB-MG-3-2	June 4, 2016	Mungyeong, Gyeongbuk	MG554263	*Monascus* sp. (HQ214967.1)	100
GB-MG-3-1	June 4, 2016	Mungyeong, Gyeongbuk	MG554262	*Polyporales* sp. (JQ312163.1)	98
GB-SJ-3-3	June 4, 2016	Sangju, Gyeongbuk	MG554242	*Cladosporium* sp. (KJ957785.1)	100
GB-SJ-3-1	June 4, 2016	Sangju, Gyeongbuk	MG554240	*Rhizopus oryzae* (JQ745257.1)	100
GB-SJ-3-2	June 4, 2016	Sangju, Gyeongbuk	MG554241	*Rhizopus oryzae* (AB109755.1)	100
GB-YC-3-2	June 4, 2016	Yecheon, Gyeongbuk	MG554237	*Aspergillus flavus* (KR611590.1)	99
GB-YC-3-1	June 4, 2016	Yecheon, Gyeongbuk	MG554236	*Aspergillus* sp. (KJ863514.1)	99
GG-YJ-3-1	June 4, 2016	Yeoju, Gyeonggi	MG554253	*Aspergillus* sp. (HQ832844.1)	99
GG-YJ-3-4	June 4, 2016	Yeoju, Gyeonggi	MG554256	*Aspergillus* sp. (KY086232.1)	100
GG-YJ-3-3	June 4, 2016	Yeoju, Gyeonggi	MG554255	*Lichtheimia ramosa* (KP132378.1)	100
GG-YJ-3-2	June 4, 2016	Yeoju, Gyeonggi	MG554254	*Mucor racemosus* (JF723566.1)	100
GN-HY-3-1	June 4, 2016	Hamyang, Gyeongnam	MG554257	*Cladosporium* sp. (KT826674.1)	100
GN-HY-3-2	June 4, 2016	Hamyang, Gyeongnam	MG554258	*Rhizopus oryzae* (JQ745257.1)	99
JJ-JJ-3-2	June 4, 2016	Jeju, Jeju	MG554239	*Chaetomium globosum* (KM268670.1)	99
JJ-JJ-3-1	June 4, 2016	Jeju, Jeju	MG554238	*Irpex lacteus* (JX290570.1)	99
JB-GC-3-3	June 4, 2016	Gochang, Jeonbuk	MG554245	*Aspergillus flavus* (EF409804.1)	99
JB-GC-3-2	June 4, 2016	Gochang, Jeonbuk	MG554244	*Aspergillus oryzae* (KX462757.1)	100
JB-GC-3-1	June 4, 2016	Gochang, Jeonbuk	MG554243	*Cladosporium* sp. (KX757230.1)	100
JB-GC-3-5	June 4, 2016	Gochang, Jeonbuk	MG554247	*Cladosporium* sp. (KJ957785.1)	100
JB-GC-3-4	June 4, 2016	Gochang, Jeonbuk	MG554246	*Penicillium citrinum* (KY921947.1)	100
JB-IK-3-2	June 4, 2016	Iksan, Jeonbuk	MG554267	*Aspergillus flavus* (GQ370372.1)	99
JB-IK-3-1	June 4, 2016	Iksan, Jeonbuk	MG554266	*Cladosporium cycadicola* (KJ869122.1)	99
JB-IM-3-1	June 4, 2016	Imsil, Jeonbuk	MG554261	*Cladosporium* sp. (KT329207.1)	100
JN-GJ-3-1	June 4, 2016	Gangjin, Jeonnam	MG554248	*Hyphopichia burtonii* (DQ683006.1)	99
JN-GJ-3-2	June 4, 2016	Gangjin, Jeonnam	MG554249	*Penicillium* sp. (HE608805.1)	99
JN-GR-3-1	June 4, 2016	Gurye, Jeonnam	MG554252	*Irpex lacteus* (KU761586.1)	99
JN-NJ-3-2	June 4, 2016	Naju, Jeonnam	MG554269	*Aspergillus flavus* (MF377553.1)	100
JN-NJ-3-1	June 4, 2016	Naju, Jeonnam	MG554268	*Aspergillus* sp. (KC834792.1)	100
JN-YG-3-2	June 4, 2016	Yeonggwang, Jeonnam	MG554228	*Aspergillus candidus* (JQ781823.1)	99
JN-YG-3-5	June 4, 2016	Yeonggwang, Jeonnam	MG554231	*Aspergillus flavus* (KR611590.1)	100
JN-YG-3-3	June 4, 2016	Yeonggwang, Jeonnam	MG554229	*Aspergillus* sp. (KC178662.1)	100
JN-YG-3-1	June 4, 2016	Yeonggwang, Jeonnam	MG554227	*Aspergillus sydowii* (KP131616.1)	100
JN-YG-3-6	June 4, 2016	Yeonggwang, Jeonnam	MG554232	*Cladosporium* sp. (HQ166315.1)	100
JN-YG-3-4	June 4, 2016	Yeonggwang, Jeonnam	MG554230	*Penicillium citrinum* (KY921947.1)	99
CB-CJ-1-2	May 18, 2017	Chungju, Chungbuk	MG554302	*Aspergillus flavus* (LC133097.1)	99
CB-JC-1-5	May 18, 2017	Jincheon, Chungbuk	MG554303	*Mucor* sp. (KX099678.1)	100
CN-NS-1-1	May 18, 2017	Nonsan, Chungnam	MG554309	*Aspergillus sydowii* (KX958061.1)	100

Table 1. Continued

Isolates	Collection Date	Region, Province	GenBank Accession No.	Most closely related fungi (GenBank Accession No.)	Similarity (%)
CN-NS-1-2	May 18, 2017	Nonsan, Chungnam	MG554310	*Lichtheimia corymbifera* (KU147463.1)	100
CN-SC-1-1	May 18, 2017	Seocheon, Chungnam	MG554306	*Hyphopichia burtonii* (KY103598.1)	100
GW-GS-1-5	May 18, 2017	Goseong, Gangwon	MG554277	*Aspergillus versicolor* (AJ937749.1)	100
GW-GS-1-1	May 18, 2017	Goseong, Gangwon	MG554275	*Beauveria bassiana* (KM249032.1)	100
GW-GS-1-4	May 18, 2017	Goseong, Gangwon	MG554276	*Penicillium neoechinulatum* (AJ005481.1)	100
GB-GJ-1-1	May 18, 2017	Gyeongju, Gyeongbuk	MG554304	*Aspergillus flavus* (KY593504.1)	100
GB-GJ-1-2	May 18, 2017	Gyeongju, Gyeongbuk	MG554305	*Aspergillus* sp. (KX450911.1)	100
GB-MG-1-3	May 18, 2017	Mungyeong, Gyeongbuk	MG554279	*Cladosporium velox* (KX788192.1)	100
GB-MG-1-2	May 18, 2017	Mungyeong, Gyeongbuk	MG554278	*Hyphopichia burtonii* (EU714323.1)	100
GB-MG-1-4	May 18, 2017	Mungyeong, Gyeongbuk	MG554280	*Hyphopichia burtonii* (EU714323.1)	100
GB-MG-1-5	May 18, 2017	Mungyeong, Gyeongbuk	MG554281	*Hyphopichia burtonii* (KY103602.1)	100
GB-SJ-1-4	May 18, 2017	Sangju, Gyeongbuk	MG554285	*Cladosporium halotolerans* (KP701942.1)	100
GB-SJ-1-2	May 18, 2017	Sangju, Gyeongbuk	MG554283	*Cladosporium* sp. (KR081401.1)	100
GB-SJ-1-3	May 18, 2017	Sangju, Gyeongbuk	MG554284	*Cladosporium sphaerospermum* (KY987535.1)	100
GB-SJ-1-1	May 18, 2017	Sangju, Gyeongbuk	MG554282	*Cladosporium velox* (KX788192.1)	99
GB-US-1-3	May 18, 2017	Uisung, Gyeongbuk	MG554273	*Cladosporium* sp. (KX148680.1)	99
GB-US-1-4	May 18, 2017	Uisung, Gyeongbuk	MG554274	*Nigrospora oryzae* (KX986075.1)	100
GG-PJ-1-1	May 18, 2017	Paju, Gyeonggi	MG554314	*Aspergillus creber* (LN898694.1)	100
GN-GS-1-5	May 18, 2017	Goseong, Gyeongnam	MG554272	*Cladosporium sphaerospermum* (KP701988.1)	100
GN-GS-1-1	May 18, 2017	Goseong, Gyeongnam	MG554271	*Mortierella oligospora* (KM265101.1)	100
GN-HA-1-3	May 18, 2017	Haman, Gyeongnam	MG554311	*Cephaliophora tropica* (FJ792583.1)	99
GN-HY-1-6	May 18, 2017	Hamyang, Gyeongnam	MG554298	*Aspergillus* sp. (KX928745.1)	100
GN-HY-1-5	May 18, 2017	Hamyang, Gyeongnam	MG554297	*Beauveria bassiana* (KY682175.1)	100
GN-HY-1-1	May 18, 2017	Hamyang, Gyeongnam	MG554296	*Penicillium crustosum* (MF188258.1)	100
GN-JJ-1-4	May 18, 2017	Jinju, Gyeongnam	MG554308	*Rhizopus oryzae* (KY244030.1)	100
GN-US-1-1	May 18, 2017	Ulsan, Gyeongnam	MG554291	*Aspergillus* sp. (KX008655.1)	99
GN-US-1-3	May 18, 2017	Ulsan, Gyeongnam	MG554293	*Cochliobolus miyabeanus* (KC315937.1)	100
GN-US-1-2	May 18, 2017	Ulsan, Gyeongnam	MG554292	*Fusarium proliferatum* (MG625088.1)	100
JB-GC-1-3	May 18, 2017	Gochang, Jeonbuk	MG554316	*Aspergillus sclerotiorum* (AY373866.1)	100
JB-GC-1-1	May 18, 2017	Gochang, Jeonbuk	MG554315	*Penicillium polonicum* (KX674637.1)	100
JB-IS-1-1	May 18, 2017	Iksan, Jeonbuk	MG554294	*Fusarium oxysporum* (KY508368.1)	100
JB-IS-1-3	May 18, 2017	Iksan, Jeonbuk	MG554295	*Penicillium* sp. (KY401140.1)	100
JB-NW-1-3	May 18, 2017	Namwon, Jeonbuk	MG554300	*Aspergillus flavus* (KX912161.1)	100
JB-NW-1-1	May 18, 2017	Namwon, Jeonbuk	MG554299	*Cephaliophora tropica* (KR809561.1)	100
JB-NW-1-4	May 18, 2017	Namwon, Jeonbuk	MG554301	*Rhizopus microsporus* (KY606252.1)	99
JN-HN-1-2	May 18, 2017	Haenam, Jeonnam	MG554307	*Monascus* sp. (KY511749.1)	100
JN-NJ-1-1	May 18, 2017	Naju, Jeonnam	MG554312	*Aspergillus versicolor* (JN638793.1)	100
JN-NJ-1-2	May 18, 2017	Naju, Jeonnam	MG554313	*Aspergillus versicolor* (GU232767.1)	100
JN-YG-1-5	May 18, 2017	Yeonggwang, Jeonnam	MG554289	*Aspergillus flavus* (JQ316530.1)	100
JN-YG-1-1	May 18, 2017	Yeonggwang, Jeonnam	MG554286	*Beauveria bassiana* (KY682175.1)	100
JN-YG-1-6	May 18, 2017	Yeonggwang, Jeonnam	MG554290	*Cephaliophora tropica* (KR809561.1)	100
JN-YG-1-3	May 18, 2017	Yeonggwang, Jeonnam	MG554288	*Cladosporium* sp. (KY643766.1)	100
JN-YG-1-2	May 18, 2017	Yeonggwang, Jeonnam	MG554287	*Penicillium crustosum* (KY906188.1)	100
CB-CJ-2-1	Aug 1, 2017	Chungju, Chungbuk	MG554336	*Mucor circinelloides* (LN809028.1)	100
CB-CJ-2-2	Aug 1, 2017	Chungju, Chungbuk	MG554337	*Mucor circinelloides* (LN809028.1)	100
CB-CJ-2-3	Aug 1, 2017	Chungju, Chungbuk	MG554338	*Mucor circinelloides* (LN809028.1)	100
CN-HS-2-1	Aug 1, 2017	Hongseong, Chungnam	MG554358	*Hyphopichia burtonii* (KY103598.1)	100
CN-NS-2-1	Aug 1, 2017	Nonsan, Chungnam	MG554344	*Aspergillus sclerotiorum* (AY373866.1)	100

Table 1. Continued

Isolates	Collection Date	Region, Province	GenBank Accession No.	Most closely related fungi (GenBank Accession No.)	Similarity (%)
CN-NS-2-3	Aug 1, 2017	Nonsan, Chungnam	MG554346	*Aspergillus sclerotiorum* (AY373866.1)	100
CN-NS-2-2	Aug 1, 2017	Nonsan, Chungnam	MG554345	*Penicillium* sp. (KX148628.1)	100
CN-TA-2-1	Aug 1, 2017	Taean, Chungnam	MG554349	*Cephaliophora tropica* (KR809561.1)	100
CN-TA-2-2	Aug 1, 2017	Taean, Chungnam	MG554350	*Cephaliophora tropica* (KR809561.1)	100
CN-TA-2-3	Aug 1, 2017	Taean, Chungnam	MG554351	*Rhizopus oryzae* (AB109754.1)	100
CN-TA-2-4	Aug 1, 2017	Taean, Chungnam	MG554352	*Rhizopus oryzae* (AB109754.1)	100
GW-GN-2-1	Aug 1, 2017	Gangneung, Gangwon	MG554353	*Aspergillus flavus* (KX462773.1)	100
GW-GN-2-2	Aug 1, 2017	Gangneung, Gangwon	MG554354	*Aspergillus flavus* (MF120213.1)	100
GW-GN-2-4	Aug 1, 2017	Gangneung, Gangwon	MG554356	*Aspergillus flavus* (MF120213.1)	100
GW-GN-2-3	Aug 1, 2017	Gangneung, Gangwon	MG554355	*Fusarium equiseti* (KY963137.1)	100
GB-GR-2-1	Aug 1, 2017	Goryeong, Gyeongbuk	MG554330	*Coprinellus* sp. (MF136551.1)	100
GB-GR-2-4	Aug 1, 2017	Goryeong, Gyeongbuk	MG554331	*Coprinellus* sp. (MF136551.1)	100
GB-GJ-2-2	Aug 1, 2017	Gyeongju, Gyeongbuk	MG554319	*Alternaria alternata* (KY814634.1)	100
GB-GJ-2-3	Aug 1, 2017	Gyeongju, Gyeongbuk	MG554320	*Alternaria alternata* (KY814634.1)	100
GB-GJ-2-1	Aug 1, 2017	Gyeongju, Gyeongbuk	MG554318	*Alternaria alternata* (MF575850.1)	100
GB-MG-2-1	Aug 1, 2017	Mungyeong, Gyeongbuk	MG554334	*Hyphopichia burtonii* (KY103598.1)	99
GB-MG-2-3	Aug 1, 2017	Mungyeong, Gyeongbuk	MG554335	*Hyphopichia burtonii* (KY103602.1)	99
GB-SJ-2-4	Aug 1, 2017	Sangju, Gyeongbuk	MG554329	*Cladosporium* sp. (HQ832966.1)	100
GB-SJ-2-1	Aug 1, 2017	Sangju, Gyeongbuk	MG554328	*Cladosporium sphaerospermum* (KY859397.1)	100
GB-US-2-1	Aug 1, 2017	Uisung, Gyeongbuk	MG554317	*Trichoderma asperellum* (KY623504.1)	100
GB-YD-2-4	Aug 1, 2017	Yeongdeok, Gyeongbuk	MG554327	*Aspergillus flavus* (KX912161.1)	100
GG-GP-2-4	Aug 1, 2017	Gimpo, Gyeonggi	MG554360	*Rhizomucor pusillus* (KJ527032.1)	100
GG-HS-2-4	Aug 1, 2017	Hwaseong, Gyeonggi	MG554357	*Cunninghamella echinulata* (KX179502.1)	100
GG-YP-2-1	Aug 1, 2017	Yangpyeong, Gyeonggi	MG554365	*Trichoderma atroviride* (KY305043.1)	99
GG-YJ-2-1	Aug 1, 2017	Yeoju, Gyeonggi	MG554359	*Uncultured fungus* (GU054203.1)	99
GN-GC-2-3	Aug 1, 2017	Geochang, Gyeongnam	MG554333	*Chaetomium globosum* (MF663683.1)	100
GN-GC-2-2	Aug 1, 2017	Geochang, Gyeongnam	MG554332	*Coprinellus* sp. (MF136551.1)	100
GN-GS-2-5	Aug 1, 2017	Goseong, Gyeongnam	MG554322	*Chaetomium globosum* (KX013209.1)	100
GN-GS-2-4	Aug 1, 2017	Goseong, Gyeongnam	MG554321	*Hyphopichia burtonii* (KY103598.1)	100
GN-GS-2-7	Aug 1, 2017	Goseong, Gyeongnam	MG554323	*Monascus* sp. (KY511749.1)	100
GN-HA-2-2	Aug 1, 2017	Haman, Gyeongnam	MG554324	*Hyphopichia burtonii* (KX965648.1)	100
GN-HA-2-3	Aug 1, 2017	Haman, Gyeongnam	MG554325	*Hyphopichia burtonii* (KX965648.1)	100
GN-HA-2-4	Aug 1, 2017	Haman, Gyeongnam	MG554326	*Monascus* sp. (KY511749.1)	100
GN-SC-2-1	Aug 1, 2017	Sancheong, Gyeongnam	MG554363	*Byssochlamys spectabilis* (KC009788.1)	100
GN-US-2-3	Aug 1, 2017	Ulsan, Gyeongnam	MG554343	*Aspergillus flavus* (KX462773.1)	100
GN-US-2-2	Aug 1, 2017	Ulsan, Gyeongnam	MG554342	*Aspergillus* sp. (KX928745.1)	100
GN-US-2-1	Aug 1, 2017	Ulsan, Gyeongnam	MG554341	*Talaromyces islandicus* (JN899318.1)	100
JB-GC-2-1	Aug 1, 2017	Gochang, Jeonbuk	MG554361	*Rhizopus microsporus* (AB381937.1)	100
JB-IM-2-1	Aug 1, 2017	Imsil, Jeonbuk	MG554362	*Hyphopichia burtonii* (KY103598.1)	99
JB-NW-2-1	Aug 1, 2017	Namwon, Jeonbuk	MG554368	*Penicillium steckii* (KX674639.1)	100
JN-BS-2-2	Aug 1, 2017	Boseong, Jeonnam	MG554366	*Mucor circinelloides* (KX620480.1)	99
JN-GR-2-1	Aug 1, 2017	Gurye, Jeonnam	MG554367	*Aspergillus protuberus* (LN898712.1)	100
JN-HN-2-1	Aug 1, 2017	Haenam, Jeonnam	MG554347	*Lichtheimia hyalospora* (GQ342894.1)	100
JN-HN-2-3	Aug 1, 2017	Haenam, Jeonnam	MG554348	*Penicillium citrinum* (MF663545.1)	100
JN-NJ-2-1	Aug 1, 2017	Naju, Jeonnam	MG554364	*Penicillium steckii* (KX674639.1)	100
JN-YA-2-2	Aug 1, 2017	Yeongam, Jeonnam	MG554340	*Aspergillus terreus* (KT778597.1)	100
JN-YA-2-1	Aug 1, 2017	Yeongam, Jeonnam	MG554339	*Hyphopichia burtonii* (KY103598.1)	100

Table 2. Identification of four geni including *Aspergillus* spp., *Penicillium* spp., *Alternaria* sp. and *Fusarium* spp. using partial beta-tubulin, calmodulin, tef1 and glyceraldehyde-3-phosphate gene sequences

Isolates	GenBank Accessions				Identified species
	b-tubulin	Calmodulin	*tef1*	GAPDH	
Aspergillus spp.					
CB-BE-3-1	MH424078	MH424038	-	-	*Aspergillus flavus*
CB-JC-3-2	MH424051	MH424011	-	-	*Aspergillus flavus*
GB-MG-3-3	MH424073	MH424033	-	-	*Aspergillus flavus*
GB-MG-3-4	MH424074	MH424034	-	-	*Aspergillus flavus*
GB-YC-3-2	MH424055	MH424015	-	-	*Aspergillus flavus*
GB-YC-3-1	MH424076	MH424036	-	-	*Aspergillus flavus*
GG-YJ-3-1	MH424071	MH424031	-	-	*Aspergillus flavus*
JB-GC-3-3	MH424052	MH424012	-	-	*Aspergillus flavus*
JB-IK-3-2	MH424053	MH424013	-	-	*Aspergillus flavus*
JN-NJ-3-2	MH424065	MH424025	-	-	*Aspergillus flavus*
JN-NJ-3-1	MH424075	MH424035	-	-	*Aspergillus flavus*
JN-YG-3-5	MH424056	MH424016	-	-	*Aspergillus flavus*
JN-YG-3-3	MH424072	MH424032	-	-	*Aspergillus flavus*
CB-CJ-1-2	MH424062	MH424022	-	-	*Aspergillus flavus*
GB-GJ-1-1	MH424061	MH424021	-	-	*Aspergillus flavus*
GB-GJ-1-2	MH424079	MH424039	-	-	*Aspergillus flavus*
GN-HY-1-6	MH424080	MH424040	-	-	*Aspergillus flavus*
GN-US-1-1	MH424077	MH424037	-	-	*Aspergillus flavus*
JB-NW-1-3	MH424059	MH424019	-	-	*Aspergillus flavus*
JN-YG-1-5	MH424054	MH424014	-	-	*Aspergillus flavus*
GW-GN-2-1	MH424057	MH424017	-	-	*Aspergillus flavus*
GW-GN-2-2	MH424063	MH424023	-	-	*Aspergillus flavus*
GW-GN-2-4	MH424064	MH424024	-	-	*Aspergillus flavus*
GB-YD-2-4	MH424060	MH424020	-	-	*Aspergillus flavus*
GN-US-2-3	MH424058	MH424018	-	-	*Aspergillus flavus*
GN-US-2-2	MH424081	MH424041	-	-	*Aspergillus flavus*
JB-GC-1-3	MH424068	MH424028	-	-	*Aspergillus sclerotiorum*
CN-NS-2-1	MH424069	MH424029	-	-	*Aspergillus sclerotiorum*
CN-NS-2-3	MH424070	MH424030	-	-	*Aspergillus sclerotiorum*
JN-YG-3-1	MH424083	MH424043	-	-	*Aspergillus sydowii*
GG-YJ-3-4	MH424082	MH424042	-	-	*Aspergillus sydowii*
CN-NS-1-1	MH424084	MH424044	-	-	*Aspergillus sydowii*
GW-GS-1-5	MH424086	MH424046	-	-	*Aspergillus versicolor*
JN-NJ-1-1	MH424088	MH424048	-	-	*Aspergillus versicolor*
JN-NJ-1-2	MH424087	MH424047	-	-	*Aspergillus versicolor*
JN-YG-3-2	MH424049	MH424009	-	-	*Aspergillus candidus*
GG-PJ-1-1	MH424050	MH424010	-	-	*Aspergillus creber*
JB-GC-3-2	MH424066	MH424026	-	-	*Aspergillus oryzae*
JN-GR-2-1	MH424067	MH424027	-	-	*Aspergillus protuberus*
JN-YA-2-2	MH424085	MH424045	-	-	*Aspergillus terreus*
Penicillium spp.					
JB-GC-3-4	MH423997	MH423985	-	-	*Penicillium citrinum*
JN-YG-3-4	MH423998	MH423986	-	-	*Penicillium citrinum*
JN-HN-2-3	MH423999	MH423987	-	-	*Penicillium citrinum*
GN-HY-1-1	MH424001	MH423989	-	-	*Penicillium crustosum*

Table 2. Continued

Isolates	GenBank Accessions				Identified species
	b-tubulin	Calmodulin	*tef1*	GAPDH	
JN-YG-1-2	MH424000	MH423988	-	-	*Penicillium crustosum*
GW-GS-1-4	MH424002	MH423990	-	-	*Penicillium neoechinulatum*
JB-GC-1-1	MH424003	MH423991	-	-	*Penicillium neoechinulatum*
JN-GJ-3-2	MH424004	MH423992	-	-	*Penicillium steckii*
JB-IS-1-3	MH424006	MH423994	-	-	*Penicillium steckii*
CN-NS-2-2	MH424005	MH423993	-	-	*Penicillium steckii*
JB-NW-2-1	MH424007	MH423995	-	-	*Penicillium steckii*
JN-NJ-2-1	MH424008	MH423996	-	-	*Penicillium steckii*
Alternaria sp.					
CN-GJ-3-2	MH423922	-	-	MH423917	*Alternaria alternata*
CN-GJ-3-1	MH423921	-	-	MH423916	*Alternaria alternata*
GB-GJ-2-2	MH423924	-	-	MH423919	*Alternaria alternata*
GB-GJ-2-3	MH423925	-	-	MH423920	*Alternaria alternata*
GB-GJ-2-1	MH423923	-	-	MH423918	*Alternaria alternata*
Fusarium sp.					
GW-GN-2-3	-	-	MH423915	-	*Fusarium equiseti*
JB-IS-1-1	-	-	MH423914	-	*Fusarium oxysporum*
GN-US-1-2	-	-	MH423913	-	*Fusarium proliferatum*

in Jeonbuk, YG, NJ, Yeongam (YA) and Gurye (GR) in Jeonnam) (Fig. 2D, Table 1 and Table 2).

In addition, genus *Penicillium*, which is known to produce ochratoxin, was also isolated from the beetles collected in 10 RPCs (GS in Kangwon province, NS in Chungnam, Iksan (IK), GC, NS in Jeonbuk, HY in Gyeongnam, YG, NJ, Gangjin (GJ), and Haenam (HN) in Jeonnam). Another toxigenic genus, *Alternaria alternata* (Ostry, 2008), was found in Gongju (GJ) in Chungnam and Gyeongju (GJ) in Gyeongbuk province. Only three *Fusarium* species including *Fusarium equiseti* (Gangneung (GN) in Gangwon province), *Fusarium oxysporum* (Iksan (IK) in Jeonbuk), and *Fusarium* sp. (Ulsan (US) from Gyeongnam) were collected in 3 RPCs (Fig. 2E, Table 1). Other fungi were identified as saprophiles that proliferate on wood and debris in the facility.

The fungi *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and *Alternaria* spp. are the major fungal species found in stored grains (Lee et al., 2011; Lee et al., 2014). More than 25% of stored grains worldwide have been reported to be contaminated with mycotoxins produced by these fungal species, and over 300 fungal metabolites have been reported to have toxicity on humans and animals (Galvano et al., 2001).

The genera *Fusarium* and *Alternaria* are known to mainly infect ears of cereal plants in the field, whereas the genera *Aspergillus* and *Penicillium* are contaminants of stored seeds, grains, and processed foods and produce mycotoxins (Adams, 1977). In particular, a number of harmful mycotoxins, such as deoxynivalenol (DON) and nivalenol (NIV), produced by *Fusarium* spp., and Aflatoxin produced by *Aspergillus* spp. are detected in stored grains (Lee et al., 2011; Lee et al., 2014; Son et al., 2011).

Both *Aspergillus flavus* and *Fusarium* spp. are known to produce mycotoxins but only *Aspergillus flavus* was found in this study. It is known that pests and fungi tend to co-occur in stored grains (Simpanya et al., 2001). It is necessary to investigate the distribution of pests and fungi in grain warehouses because pests promote the growth and propagation of fungi.

According to the studies on fungal and mycotoxin contamination of RPC grain samples, *Aspergillus* and *Penicillium* species were infrequently found nationwide but were particularly abundant in a few RPC samples (Lee et al., 2014). *Alternaria*, *Nigrospora*, and *Epicoccum* species were more consistently isolated at similar frequencies, whenever fungal contamination was detected. In accordance with the results from previous studies (Lee et al., 2014; Son et al., 2011), genera *Aspergillus*, *Penicillium*, *Alternaria*, and *Nigrospora* were identified from the *T. castaneum* collected at RPCs. Therefore, it is suspected that the red flour beetles are a potential vector for the transfer of toxigenic fungi and mycotoxins.

According to the study on mycotoxin contamination in different growth stages of rice (Nakaijima et al., 2008; Nash and Snyder, 1962), rice plants are always exposed to fungi and mycotoxins even before storage. So far, it has been reported that differences in temperature and humidity depending on the climate have a great influence on the growth of fungi and occurrence of mycotoxins (Russell et al., 2010). However, studies on the effect of temperature and humidity on pest-assisted mycotoxin production in stored grains are uncommon and remained to be investigated in the future. Our study shows that the storage pest, *T. castaneum*, could play an important role in transmission of fungi in stored rice in RPC and potentially contribute to mycotoxin contamination of rice.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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