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%), *Cephalophora* spp. (3.5%), *Alternaria alternata* (2.8%) and *Monascus* sp. (2.8%). Less commonly identified were genera *Fusarium*, *Nigrospora*, *Beauveria*, *Chaetomium*, *Coprinellus*, *Irplex*, *Lichtheimia*, *Trichoderma*, *Byssochlamys*, *Cochliobolus*, *Cunninghamamella*, *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). Degrad-
tion 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 (Tipple, 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 B1 (AFB1), fumonisin B1 (FB1), 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, 1995; 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 (Bioneer, 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).
tained 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* sp. (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%), *Cephalophora 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.](image-url)
were Fusarium spp., Beauveria bassiana, Chaetomium globosum, Coprinellus sp., Irpex lacteus, Lichtheimia spp., Trichoderma spp., Byssoschlamys 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 from 16 RPCs (Gangneung (GN) in Gangwon (GW) province, Yeoju (YJ) in Gyeonggii, Jinju (JC), Changju (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 Gyeonggii, 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. (KX462757.1) | 100 |
| GB-MG-3-2 | June 4, 2016 | Mungyeong, Gyeongbuk | MG554262 | Monascus sp. (HQ312163.1) | 98 |
| GB-MG-3-1 | June 4, 2016 | Mungyeong, Gyeongbuk | MG554263 | 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-3-3 | June 4, 2016 | Yeoju, Gyeonggi | MG554255 | Lichtheimia ramosa (KP132378.1) | 100 |
| GG-YJ-3-4 | June 4, 2016 | Yeoju, Gyeonggi | MG554256 | Cladosporium sp. (JQ745257.1) | 100 |
| GB-IM-3-1 | June 4, 2016 | Imsil, Jeonbuk | MG554239 | Aspergillus flavus (JF723566.1) | 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 (GQ730732.1) | 99 |
| JB-IK-3-1 | June 4, 2016 | Iksan, Jeonbuk | MG554266 | Cladosporium cycadica (KJ869122.1) | 99 |
| JB-IM-3-1 | June 4, 2016 | Imsil, Jeonbuk | MG554261 | Cladosporium sp. (KT329207.1) | 100 |
| JN-GR-3-1 | June 4, 2016 | Gurye, Jeonnam | MG554252 | Irpex lacteus (KU761586.1) | 99 |
| JN-JJ-3-2 | June 4, 2016 | Jeju, Jeju | MG554259 | Aspergillus flavus (MF377553.1) | 100 |
| JN-GR-3-2 | June 4, 2016 | Gurye, Jeonnam | MG554255 | Aspergillus flavus (MF377553.1) | 100 |
| JN-JG-3-2 | June 4, 2016 | Naju, Jeonnam | MG554268 | Aspergillus flavus (KX347921.1) | 99 |
| JN-JG-3-1 | June 4, 2016 | Naju, Jeonnam | MG554265 | Anguillulae sp. (KX347921.1) | 99 |
| JN-JG-3-3 | June 4, 2016 | Naju, Jeonnam | MG554264 | Aspergillus candidus (JQ781823.1) | 99 |
| JN-JG-3-5 | June 4, 2016 | Naju, Jeonnam | MG554263 | Aspergillus flavus (KR611590.1) | 99 |
| JN-JG-3-3 | June 4, 2016 | Naju, Jeonnam | MG554262 | Aspergillus flavus (KX347921.1) | 99 |
| JN-JG-3-4 | June 4, 2016 | Naju, Jeonnam | MG554260 | Aspergillus flavus (KX347921.1) | 99 |
| JN-JG-3-2 | June 4, 2016 | Yeonggwang, Jeonnam | MG554228 | Aspergillus flavus (LC133097.1) | 99 |
| JN-JG-3-1 | June 4, 2016 | Yeonggwang, Jeonnam | MG554227 | Aspergillus sydowii (KP131616.1) | 100 |
| JN-JG-3-6 | June 4, 2016 | Yeonggwang, Jeonnam | MG554232 | Cladosporium sp. (HQ166315.1) | 100 |
| JN-JG-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. (KX909678.1) | 100 |
| CN-NS-1-1 | May 18, 2017 | Nonsan, Chungnam | MG554309 | Aspergillus sydowii (KX958061.1) | 100 |
| 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             | *Aspergillus flavus* (KY103602.1)                | 100          |
| GB-SJ-1-1 | May 18, 2017    | Seocheon, Chungnam  | MG554271             | *Mortierella oligospora* (LN898694.1)           | 100          |
| GB-SJ-1-3 | May 18, 2017    | Seocheon, Chungnam  | MG554272             | *Cladosporium sphaerospermum* (FP792583.1)       | 99           |
| GB-SJ-1-5 | May 18, 2017    | Seocheon, Chungnam  | MG554273             | *Cladosporium sp.* (KX148680.1)                  | 99           |
| GB-US-1-3 | May 18, 2017    | Ulsan, Gyeongnam   | MG554274             | *Nigrospora oryzae* (KX986075.1)                 | 100          |
| GB-US-1-4 | May 18, 2017    | Ulsan, Gyeongnam   | MG554275             | *Aspergillus creber* (KX928745.1)                | 100          |
| JN-HN-1-2 | May 18, 2017    | Jeonju, Jeonbuk    | MG554301             | *Rhizopus oryzae* (KY244030.1)                   | 100          |
| JN-HN-1-3 | May 18, 2017    | Jeonju, Jeonbuk    | MG554302             | *Cephalophora tropica* (FJ792583.1)              | 99           |
| JN-HY-1-5 | May 18, 2017    | Hamyang, Gyeongnam | MG554297             | *Beauveria bassiana* (KX682175.1)                | 100          |
| JN-HY-1-6 | May 18, 2017    | Hamyang, Gyeongnam | MG554296             | *Penicillium crustosum* (MF188258.1)             | 100          |
| JN-JJ-1-4 | May 18, 2017    | Jinju, Gyeongnam   | MG554308             | *Rhizopus oryzae* (KY244030.1)                   | 100          |
| JN-JJ-1-3 | May 18, 2017    | Jinju, Gyeongnam   | MG554295             | *Penicillium sp.* (KY401140.1)                   | 100          |
| JN-NJ-1-1 | May 18, 2017    | Naju, Jeonbuk      | MG554311             | *Cephalophora tropica* (FJ792583.1)              | 99           |
| JN-NJ-1-2 | May 18, 2017    | Naju, Jeonbuk      | MG554302             | *Aspergillus sp.* (KX008655.1)                   | 99           |
| JN-YG-1-1 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554299             | *Cladosporium velox* (KX788192.1)               | 99           |
| JN-YG-1-2 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554300             | *Cladosporium velox* (KX912161.1)                | 100          |
| JN-YG-1-3 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554301             | *Fusarium proliferatum* (MG625088.1)            | 100          |
| JN-YG-1-6 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554299             | *Fusarium oxysporum* (KY508368.1)                | 100          |
| JN-YG-1-5 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554295             | *Penicillium sp.* (KY401140.1)                   | 100          |
| JN-YG-1-4 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554300             | *Penicillium velox* (KX912161.1)                 | 100          |
| JN-YG-1-3 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554301             | *Rhizopus oryzae* (KY244030.1)                   | 100          |
| JN-YG-1-2 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554302             | *Cephalophora tropica* (KX912161.1)              | 100          |
| JN-YG-1-1 | May 18, 2017    | Yeonggwang, Jeonbuk | MG554301             | *Aspergillus flavus* (KX912161.1)                | 100          |
| 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              | Cephalophora tropica (KR809561.1)                  | 100            |
| CN-TA-2-2   | Aug 1, 2017     | Taean, Chungnam    | MG554350              | Cephalophora 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            |
| GB-GR-2-1   | Aug 1, 2017     | Goryeong, Gyeongbuk| MG554355              | Fusarium equiseti (KY963137.1)                     | 100            |
| GB-GR-2-4   | Aug 1, 2017     | Goryeong, Gyeongbuk| MG554357              | Alternaria alternata (KX814634.1)                  | 100            |
| GB-SJ-2-1   | Aug 1, 2017     | Gyeongju, Gyeongbuk| MG554359              | Alternaria alternata (MF575850.1)                  | 100            |
| GB-US-2-1   | Aug 1, 2017     | Ulsan, Gyeongnam   | MG554361              | Trichoderma asperellum (KY623504.1)                | 100            |
| GB-YD-2-4   | Aug 1, 2017     | Yeongdeok, Gyeongbuk| MG554362             | 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 burtoni (KY103598.1)                   | 99             |
| 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 burtoni (KX965648.1)                   | 100            |
| GN-HA-2-3   | Aug 1, 2017     | Haman, Gyeongnam   | MG554325              | Hyphopichia burtoni (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              | Byssoschlamys 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 flavus (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 burtoni (KY103598.1)                   | 99             |
| JB-NW-2-1   | Aug 1, 2017     | Namwon, Jeonbuk    | MG554368              | Penicillium steckii (KX674639.1)                   | 100            |
| JB-US-2-2   | Aug 1, 2017     | Boseong, Jeonnam   | MG554366              | Mucor circinelloides (KX620480.1)                  | 99             |
| JB-NR-2-1   | Aug 1, 2017     | Gurye, Jeonnam     | MG554367              | Aspergillus protuberus (LN899712.1)                | 100            |
| JB-HN-2-1   | Aug 1, 2017     | Haenam, Jeonnam    | MG554347              | Lichtheimia hyalospora (GQ342894.1)                | 100            |
| JB-HN-2-3   | Aug 1, 2017     | Haenam, Jeonnam    | MG554348              | Penicillium citrinum (MF663545.1)                  | 100            |
| JB-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 burtoni (KY103598.1)                   | 100            |
| Isolates  | GenBank Accessions | Identified species       |
|-----------|--------------------|-------------------------|
| **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.** 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.
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* (Ik-san (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; 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

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**Table 2. Continued**

| Isolates | GenBank Accessions | Identified species |
|----------|--------------------|-------------------|
|          | b-tubulin | Calmodulin | tefl | 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* |
| 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 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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