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The Proportion of Non-Aflatoxigenic Strains of the *Aspergillus flavus/oryzae* Complex from Meju by Analyses of the Aflatoxin Biosynthetic Genes[§]

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Strains of the *Aspergillus flavus/oryzae* complex are frequently isolated from meju, a fermented soybean product, that is used as the starting material for ganjang (soy sauce) and doenjang (soybean paste) production. In this study, we examined the aflatoxin producing capacity of *A. flavus/oryzae* strains isolated from meju. 192 strains of *A. flavus/oryzae* were isolated from more than 100 meju samples collected from diverse regions of Korea from 2008 to 2011, and the *norB-cypA*, *omtA*, and *aflR* genes in the aflatoxin biosynthesis gene cluster were analyzed. We found that 178 strains (92.7%) belonged to non-aflatoxigenic group (Type I of *norB-cypA*, IB-L-B-, IC-AO, or IA-L-B- of *omtA*, and AO type of *aflR*), and 14 strains (7.3%) belonged to aflatoxin-producible group (Type II of *norB-cypA*, IC-L-B+/B- or IC-L-B+ of *omtA*, and AF type of *aflR*). Only 7 strains (3.6%) in the aflatoxin-producible group produced aflatoxins on Czapek yeast-extract medium. The aflatoxin-producing capability of *A. flavus/oryzae* strains from other sources in Korea were also investigated, and 92.9% (52/56) strains from air, 93.9% (31/33) strains from rice straw, 91.7% (11/12) strains from soybean, 81.3% (13/16) strains from corn, 82% (41/50) strains from peanut, and 73.2% (41/56) strains from arable soil were included in the non-aflatoxigenic group. The proportion of non-aflatoxigenicity of meju strains was similar to that of strains from soybean, air and rice straw, all of which have an effect on the fermentation of meju. The data suggest that meju does not have a preference for non-aflatoxigenic or aflatoxin-producible strains of *A. flavus/oryzae* from the environment of meju. The non-aflatoxigenic meju strains are proposed to be named *A. oryzae*, while the meju strains that can produce aflatoxins should be referred to *A. flavus* in this study.

***Keywords*:** aflatoxin, *Aspergillus flavus*, *Aspergillus flavus/oryzae* complex, *Aspergillus oryzae*, Meju

Introduction

Meju is a brick of dried fermented soybean that serves as the starting material in the production of doenjang (soybean paste) and ganjang (soy sauce), which are essential ingredients in authentic Korean cuisine. Meju is naturally fermented by various microorganisms such as bacteria, yeasts, and fungi. Fungi play an important role in the process of fermentation and degrade the macromolecules present in soybeans into micronutrients (Lee, 1995). *Aspergillus flavus/oryzae* complex (*A. flavus/oryzae*) that cannot be differentiated by conventional taxonomic criteria, is frequently isolated from naturally fermented meju. The fungal species in meju has attracted considerable attention, because *A. oryzae* is used as a fermentation agent for Japanese miso and shoyu (Kitamoto, 2002), whereas *A. flavus* produce aflatoxins that are the most toxic ones among the known carcinogenic substances (Yu *et al*., 2004).

It is suggested that *A. flavus* in meju may be a culprit of the high incidence of stomach cancer among Koreans by the Time article (1969, http://www.time.com/time/magazine/article/0,9171,844829,00.html). Kim *et al.* (2001) reported that aflatoxin B1 was found in 35 of 60 (58.3%) meju samples at an average concentration on 7.3 ng/g by ELISA, and aflatoxin B1 contamination was confirmed in 25 of 60 samples (41.6%) by high-performance liquid chromatography (HPLC), with an average concentration of 6.9 ng/g. Park *et al.* (2001) reported that 6 of 24 *Aspergillus* strains isolated from the commercial meju from the western Gyeongnam province of Korea, produced aflatoxins on Sucrose low salts (SLS) media. Kim *et al.* (2011) also reported that 4 of 65 *Aspergillus* strains from meju collected from 2009 to 2010, produced aflatoxins on yeast extract sucrose (YES) agar. However, Park and Lee (1987, 1989) and Park *et al.* (1988, 2003) demonstrated that the aflatoxins can be destroyed by NH_3, sunlight, and other mixed culture conditions during the preparation of ganjang and doenjang. Hwang *et al.* (2008) reported that aflatoxin B_1 in meju decreased by 3%

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and 80% during the fermentation of ganjang and doenjang, respectively.

It is known that non-aflatoxigenic strains have one or more mutations and/or deletions in regions containing the aflatoxin biosynthesis gene cluster (Chang *et al.*, 2005). Chang *et al.* (2006) reported that single nucleotide polymorphisms in the *omtA* gene and deletions in the *norB-cypA* region would provide a basis for the selection of non-aflatoxigenic *A. oryzae/flavus* isolates for use in industrial fermentation. In general, L-type isolates with Type II *norB-cypA* deletion have relatively stable aflatoxin-producing ability, whereas Type I deletion is associated with *A. oryzae* isolates, atoxigenic L-type *A. flavus* isolates, and toxigenic S-type *A. flavus* isolates. Lee *et al.* (2006) reported that the *aflR* gene from *A. oryzae* can be distinguished from that in *A. flavus* by two amino acid substitutions. The *aflR* mutation occurring in *A. oryzae* may significantly change protein activity and eliminate the normal function of the AflR protein. Hence, they postulated that AO type strains of *aflR* are non-aflatoxigenic, although they were identified as *A. flavus*.

In this study, we examined the *norB-cypA*, *omtA*, and *aflR* genes in the aflatoxin biosynthesis gene cluster in order to elucidate the aflatoxin-producing potential of *A. flavus/oryzae* strains from meju, and compared the results with *A. flavus/oryzae* strains isolated from other sources such as soybean, rice straw, air, soil, peanut, and corn in Korea.

Table 1. **Representative *A. flavus/oryzae* complex strains isolated from meju used in this study**

Strain no. (KACC[a])	Scientific name	Source	*norB-cypA*		*omtA*	*aflR*	Aflatoxin (B1)
			PCR no.[d]	Type			
46449	*A. flavus*	Meju, Juwon, Korea	113	II	IC-L-B+	AF	O
46450	*A. flavus*	Meju, Gimcheon, Korea	89	II	IC-L-B+	AF	X
46451	*A. flavus*	Meju, Icheon, Korea	90	II	IC-L-B+	AF	X
46452	*A. flavus*	Meju, Icheon, Korea	97	II	IC-L-B+	AF	X
46453	*A. flavus*	Meju, Buan, Korea	173	II	IC-L-B+	AF	O
46812	*A. flavus*	Meju, Jeongeup, Korea	24	II	IC-L-B+	AF	X
46813	*A. flavus*	Meju, Juwon, Korea	109	II	IC-L-B+	AF	X
46814	*A. flavus*	Meju, Juwon, Korea	111	II	IC-L-B+	AF	X
46447	*A. flavus*	Meju, Jeongeup, Korea	26	II	IC-L-B+/B-	AF	O
46448	*A. flavus*	Meju, Hapcheon, Korea	53	II	IC-L-B+/B-	AF	O
46454	*A. flavus*	Meju, Goisan, Korea	143	II	IC-L-B+/B-	AF	O
46815	*A. flavus*	Meju, Jeongeup, Korea	25	II	IC-L-B+/B-	AF	O
46816	*A. flavus*	Meju, Anseong, Korea,	117	II	IC-L-B+/B-	AF	X
46817	*A. flavus*	Meju, Yongin, Korea	147	II	IC-L-B+/B-	AF	O
46810	*A. oryzae*	Meju, Sunchang, Korea	55	X	IA-L-B-	X	X
46811	*A. oryzae*	Meju	56	X	IA-L-B-	X	ND[e]
46457	*A. oryzae*	Meju, Goisan, Korea	48	I	IA-S-B+	X	X
46455	*A. oryzae*	Meju, Gyeongsan, Korea	1	I	IB-L-B-	Ao	X
46458	*A. oryzae*	Meju, Yeoju, Korea	104	I	IB-L-B-	Ao	X
46460	*A. oryzae*	Meju	98	I	IB-L-B-	Ao	X
46461	*A. oryzae*	Meju, Anseong, Korea,	157	I	IB-L-B-	Ao	ND
46462	*A. oryzae*	Meju, Goisan, Korea	163	I	IB-L-B-	Ao	ND
46468	*A. oryzae*	Meju, Yangpyeong, Korea	185	I	IB-L-B-	Ao	ND
46470	*A. oryzae*	Meju, Yongin, Korea	127	I	IB-L-B-	Ao	ND
46471	*A. oryzae*	Meju, Icheon, Korea	131	X	IB-L-B-	Ao	X
46472	*A. oryzae*	Meju, Gongju, Korea	148	I	IB-L-B-	Ao	ND
46473	*A. oryzae*	Meju, Goisan, Korea	151	I	IB-L-B-	Ao	ND
46456	*A. oryzae*	Meju, Haenam, Korea	17	I	IC-Ao	Ao	ND
46459	*A. oryzae*	Meju	65	I	IC-Ao	Ao	X
46463	*A. oryzae*	Meju, Goryeong, Korea	167	I	IC-Ao	Ao	ND
46467	*A. oryzae*	Meju, Gyeongsan, Korea	181	I	IC-Ao	Ao	ND
46469	*A. oryzae*	Almeju, Korea	190	I	IC-Ao	Ao	ND
46474	*A. oryzae*	Meju, Damyang, Korea	153	I	IC-Ao	Ao	ND
46465	*A. oryzae*	Meju, Buan, Korea	174	I	X	Ao	ND
46466	*A. oryzae*	Meju, Gongju, Korea	176	I	X	X	ND
CBS[b] 100927[NT]	*A. flavus*	cellophan, South pacific		II	IC-L-B+/B-	AF	O
CBS 466.91[T]	*A. oryzae*	unknown, Osaka, Japan		I	IB-L-B-	ND	X
RIB[c] 40	*A. oryzae*	cereal grain, Kyoto, Japan		I	IB-L-B-	Ao	X

[a] KACC: Korean Agricultural Culture Collection, Suwon, Korea.
[b] CBS: CBS Fungal Biodiversity Center, Utrecht, Netherlands.
[c] RIB: National Research Institute of Brewing, Osaka, Japan.
[d] The numbers represent the lane numbers shown on top of the agarose gel electrophoresis data in Fig. 1.
[e] ND: Not determined.

Materials and Methods

Isolation of *A. flavus/oryzae* strains from meju and other sources

We isolated 156 *A. flavus/oryzae* strains from 98 meju loaves, which were collected from various regions in Korea [Gangwon (n=6), Gyeonggi (n=30), Gyeongnam (n=3), Gyeongbuk (n=15), Jeonnam (n=5), Jeonbuk (n=28), Jeju (n=4), Chungnam (n=3), and Chungbuk (n=4)] from 2008 to 2011. Two isolation methods, namely, direct plating and dilution plating (Hong *et al.*, 2011) were used. Additionally, we visited diverse meju farms in Chungnam, Chungbuk, Jeonnam, Jeonbuk, and Gyeongbuk provinces in mid February 2011, and we isolated 36 *A. flavus/oryzae* strains from meju loaves by direct examination and plating of the fungi on malt extract agar (MEA; 50 g MEA [Oxoid CM0059] in 1 L DW) (Table 1 and Supplementary data Table S1).

Additionally, 223 *A. flavus/oryzae* strains were isolated from various other sources, including soil, air, corn, peanut, soybean, and rice straw, in Korea as follows: (1) 56 *A. flavus/oryzae* strains were isolated from arable soils of Gwangju (a metropolitan city), Daegu, Siheung, Suwon, Namhae, Suncheon, Hwasun, and Jeju by the method described by Ehrlich *et al.* (2003); (2) 56 *A. flavus/oryzae* strains were collected either from the indoor or outdoor air in Suwon, Bucheon, Seoul, Yangpeong, and Anyang by using Merck MAS-100 air samplers (USA) with dichloran rose bengal chlorampenicol (DRBC; Oxoid) and Dichloran glycerol 18 (DG18; Oxoid); (3) 50 *A. flavus/oryzae* strains were isolated from various peanuts obtained from the National Agrobiodiversity Center in Suwon by direct plating methods on MEA, DG18, and DRBC; (4) 33 *A. flavus/oryzae* strains were isolated from rice straws by direct plating on DG18 and DRBC in Yangyang, Buan, Haenam, Gongju, Sunchang, Icheon, Anseong, Yangpeong, and Gangjin; (5) 12 *A. flavus/oryzae* strains were isolated from soybeans in Icheon, Gongju, Sunchang, Anseong, Chilgok, Shiheung, by direct plating on MEA, DG18, and DRBC; and (6) 16 *A. flavus/oryzae* strains were isolated from corns from Hongcheon by direct plating on MEA, DG18, and DRBC. All fungal cultures were stored on MEA slants at 4°C and by spore suspension in 0.1% (v/v) Tween 80 at -20°C.

Analyses of the aflatoxin biosynthesis genes: *norB-cypA*, *omtA*, and *aflR*

Spore suspensions of the total 415 *A. flavus/oryzae* strains from meju and other sources were inoculated in malt extract broth (Oxoid CM57), and mycelia were harvested. Genomic DNA was extracted by DNeasy Plant Mini Kit (Qiagen 69106) according to the manufacturer's instructions and maintained at -20°C. PCR amplification of the *norB-cypA* region of *A. flavus/oryzae* strains were performed using the primer pair 5′-AGTTGCGATCTGTAACACTGCTGA-3′ and 5′-GGAACGGGTCAAGGATATAAGGG-3′ by the method described by Ehrlich *et al.* (2004). Amplified products were identified on 1.2% agarose gel. The *omtA* gene of *A. flavus/oryzae* strains were amplified and sequenced using the primer pair 5′-CAGGATATCATTGTGGACGG-3′ and 5′-CTCCTCTACCAGTGGCTTCG-3′ by the method described by Geiser *et al.* (1998), and the reference sequences used were obtained from Chang *et al.* (2006). The *aflR* gene of 192 meju strains were amplified and sequenced with the following pair of primers: F2 (5′-CCGGCGCATAACACG TACTC′-3) and R2 (5′-GGCGCTTGGCCAATAGGTTC-3′) by the method described by Lee *et al.* (2006), and the reference sequences used were those described by Lee *et al.* (2006). The sequences were analyzed using the Tamura-Nei parameter distance calculation model, which was then used to construct the neighbor-joining tree with MEGA version 5.10 (Tamura *et al.*, 2011).

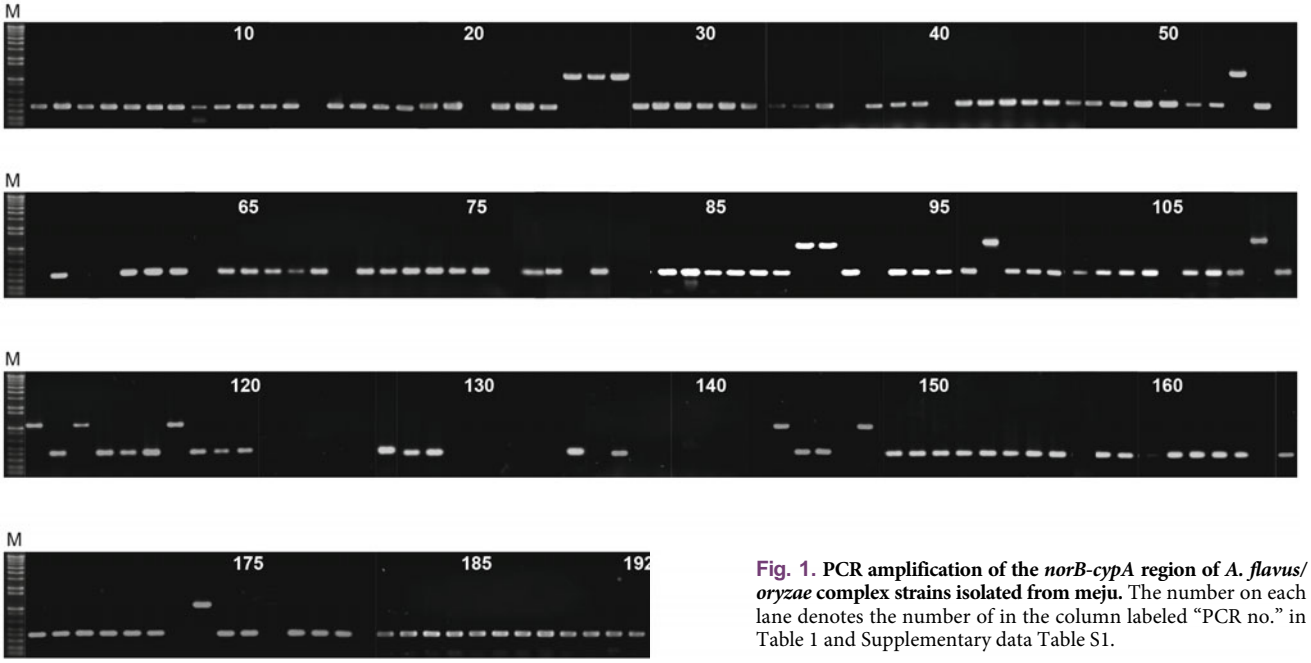

Fig. 1. PCR amplification of the *norB-cypA* region of *A. flavus/oryzae* complex strains isolated from meju. The number on each lane denotes the number of in the column labeled "PCR no." in Table 1 and Supplementary data Table S1.

Analyses of aflatoxin production

Sixty-eight strains which were selected from the 178 non-aflatoxigenic group (Type I of *norB-cypA*, IB-L-B-, IC-AO, or IA-L-B- of *omtA*, and AO type of *aflR*) and 14 strains that belonged to the aflatoxin-producible group (Type II of *norB-cypA*, IC-L-B+/B- or IC-L-B+ of *omtA*, and AF type of *aflR*) were used for aflatoxin production analyses (Supplementary data Table S1). The production of aflatoxins by the selected strains was determined cultural and HPLC analyses as described by Jung *et al*. (2012). Briefly, a 0.1 ml aliquot of individual fungal spore suspension was used to inoculate into 50 ml of sterile Czapek yeast-extract (CY; $NaNO_3$ 3 g, KH_2PO_4 1 g, KCl 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, $FeSO_4{\cdot}H_2O$ 0.01 g, yeast extract 5 g, sucrose 30 g, and distilled water 1 L), which was used for the production of aflatoxins by the fungi. The inoculated flasks were incubated for 14 days at 25°C. The fungal culture broth was cleaned up using an immunoaffinity column (AflaTest® $_{WB}$, Vicam Co., USA), and then used for HPLC analysis. The clean-up residue was derivatized using trifluoroacetic acid, filtered through a 0.22 μm membrane filter, and injected onto HPLC. Analysis of aflatoxins from the injected 50 μl of samples was carried out using a Nova-Pack® C18 column (150 mm, 3.9 mm i.d., 4 μm, Waters, USA). The mobile phase was acetonitrile-methanol-water (17:17:66, v/v/v), pumped at a constant flow rate of 0.5 ml/min. The quantitative determination of each aflatoxin was carried out using a fluorescence detector (excitation: 360 nm; emission: 440 nm).

Results

A total of 192 *A. flavus/oryzae* meju strains were divided into 3 groups by PCR amplification of *norB-cypA* region (Fig. 1, Table 1, and Supplementary data Table S1). Of these, 144 strains produced 0.3-kb PCR fragments (Type I deletion), and 14 strains produced 0.8-kb PCR fragments (Type II deletion). The remaining 34 strains showed no amplification in *norB-cypA* region.

A. flavus/oryzae meju strains were segregated into 6 groups after analyses of the *omtA* gene (Fig. 2, Table 1, and Supplementary data Table S1): 110 strains clustered into the IB-L-B- group; 57 strains clustered into the IC-AO group; Two strains clustered into the IA-L-B- group; 8 strains were grouped under IC-L-B+/B-, 6 strains were grouped under IC-L-B+, and one strain was grouped under IA-S-B+. In the group name, L and S mean large and small sclerotium, respectively, and B+ and B- mean aflatoxin B production and non-production, respectively. The *omtA* gene from 8 strains could not be amplified with the primers used.

According to analyses of the *aflR* gene in the aflatoxin biosynthesis gene cluster, 131 meju strains clustered into the *A. oryzae* (AO) group, including the BCRC 30174, type strain of *A. oryzae,* and 14 meju strains clustered into the *A. flavus* (AF) group, including the CBS100927, type strain of *A. flavus* (Fig. 3, Table 1, and Supplementary data Table S1). The *aflR* gene could not be amplified from the 47 meju strains in this study. All 14 strains in the AF type of *aflR* gene showed Type II deletion of the *norB-cypA* region and were included in aflatoxin B-producible groups (IC-L-B+ and IC-L-B+/B-) of the *omtA* gene (Table 1).

Only seven (KACC 46449, 46453, 46815, 46447, 46448, 46454, and 46817) of the 192 *A. flavus/oryzae* meju strains produced aflatoxin B on Czapek yeast-extract (Table 1 and Supplementary data Table S1). These 7 strains showed Type II deletion of *norB-cypA* region and AF type of the *aflR* gene. Five strains of them were included in the IC-L-B+ group, and two strains of them were included in the IC-L-B+/B- group of *omtA* gene. KACC 46457 which was included in Type I deletion of *norB-cypA* region, IA-S-B+ of *omtA*, and no amplification of *aflR* gene, did not produce aflatoxin B on Czapek yeast-extract (Table 1). 68 strains that belonged to non-aflatoxigenic group (Type I of *norB-cypA*, IB-L-B-, IC-AO or IA-L-B- of *omtA*, and AO type of *aflR*) did not produce aflatoxin B (Table 1 and Supplementary data Table S1). Only the strains that harbored in Type II deletion of *norB-cypA* region, AF type of *aflR* and IC-L-B+/B- or IC-L-B+ of *omtA* had the potential to produce aflatoxin B.

Of the 223 *A. flavus/oryzae* strains isolated from air, rice straw, soybean, corn, peanut, and arable soils in Korea, 144 strains produced a 0.3-kb band (Type I deletion), 28 strains produced a 0.8-kb band (Type II deletion), 6 strains produced a 1.8-kb band, and 45 strains did not produce any band by PCR amplification of the *norB-cypA* region. The all 28 strains which showed Type II deletion of the *norB-*

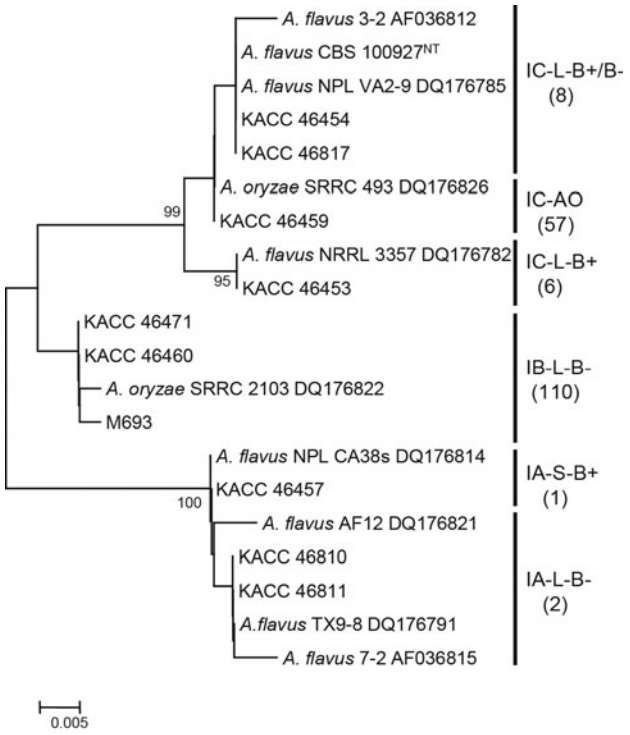

Fig. 2. Phylogenetic tree of *A. flavus/oryzae* complex strains from meju inferred from neighbor-joining analysis of the *omtA* gene sequence. The sequences of KACC strains were analyzed in this study and those of the other strains were obtained from Chang *et al.* (2006). Numbers above/below the nodes are bootstrap values (1,000 replicates, value <70% are not shown). "L" and "S" indicate size of the sclerotia and "B+" and "B-" indicate productivity of aflatoxin B.

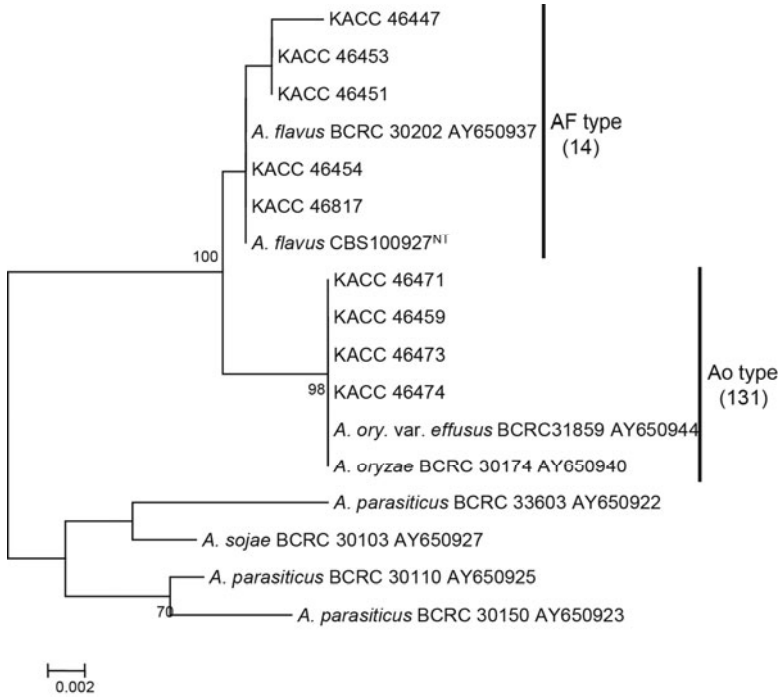

Fig. 3. Phylogenetic tree of *A. flavus/oryzae* complex strains from meju inferred from neighbor-joining analysis of the *aflR* gene sequence. The sequences of KACC strains were analyzed in this study and those of the other strains were obtained from Lee *et al.* (2006). Numbers above/below the nodes are bootstrap values (1,000 replicates, value <70% are not shown).

cypA region, clustered into IC-L-B+/B- or IC-L-B+ group in the phylogenetic analysis of *omtA*, and 26 strains of them produced aflatoxin B on Czapek yeast-extract (data not shown). All six strains which produced a 1.8-kb band, produced aflatoxin B. The number of aflatoxin-producible strains that harbored in a 0.8-kb band producer and a 1.8 kb band producer of the *norB-cypA* region, were: 4 (7.1%, n=56) from air, 2 (6.1%, n=33) from rice straw, 1 (8.3%, n=12) from soybean, 3 (18.7%, n=16) from corn, 9 (18.0%, n=50) from peanut, and 15 (26.8%, n=56) from arable soil (Fig. 4).

Discussion

Of 192 *A. flavus/oryzae* meju strains, 178 strains (92.7%) were included in the non-aflatoxigenic group (Type I of *norB-cypA*, IB-L-B-, IC-AO, or IA-L-B- of *omtA*, and AO type of *aflR*), and 68 strains selected in this study did not produce aflatoxin B (Supplementary data Table S1). Fourteen strains (7.3%) were included in the aflatoxin-producible group (Type II of *norB-cypA*, IC-L-B+/B- or IC-L-B+ of *omtA*, and AF type of *aflR*), and 7 strains (3.6%) of them produced aflatoxins on Czapek yeast-extract. Five of the 6 strains from the IC-L-B+ group of *omtA* produced aflatoxin B, but only 2 of 8 strains from the IC-L-B+/B- group of *omtA* produced aflatoxin B. Furthermore, 2 of 28 strains which are originated from other sources such as air, rice straw, soybean, corn, peanut, and arable soil, and which belonged to Type II of the *norB-cypA* region and IC-L-B+ or IC-L-B+/B- groups of *omtA*, did not produce aflatoxin B on Czapek yeast-extract. These data suggest that all strains included in the aflatoxin-producible group (Type II of *norB-cypA*, IC-L-B+/B- or IC-L-B+ of *omtA*, and AF type of *aflR*) do not produce aflatoxin. On the other hand, any strains included in the non-aflatoxigenic group do not produce aflatoxin.

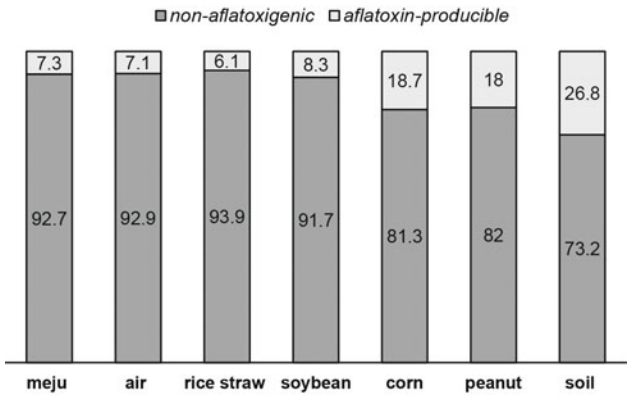

Fig. 4. Proportion of non-aflatoxigenic strains of the *A. flavus/oryzae* complex that originated from meju and other sources in Korea by evaluating aflatoxin biosynthetic genes.

We found that 92.7% of *A. flavus/oryzae* meju strains belonged to the non-aflatoxigenic group. This proportion is higher than that in corn, peanut, and arable soil, but is similar to that in air, rice straw, and soybean. Corn and peanut are known as niches for aflatoxigenic *A. flavus* (Horn, 2007), the crops might have a preference for an aflatoxigenic strain of *A. flavus*. Air and rice straw are present in the environment of meju fermentation and can provide the meju with *A. flavus/oryzae* strains during fermentation. The result suggests that meju does not have a preference for non-aflatoxigenic or aflatoxin-producible strains of *A. flavus/oryzae* from the environment of meju.

The proportion of strains from a non-aflatoxigenic population of *A. flavus* obtained from the different parts of the world varies considerably. Approximately 40% of naturally

occurring strains of *A. flavus* in the Unites States lack the ability to produce aflatoxin (Cary and Ehrlich, 2006). In comparison to this, high proportions (92.7%) of *A. flavus/oryzae* strains that originate from meju lack the ability to produce aflatoxin. Meju is fermented during winter when the weather is cold, and *A. flavus/oryzae* strains used in this study were isolated from cold environment. The data therefore suggests that the high proportion of non-aflatoxigenicity of *A. flavus/oryzae* meju strains was due to prevalent low temperatures, but further studies are required to verify this hypothesis.

A. flavus/oryzae is not rare in meju environment such as air, soybean, and rice straw, and the species have been frequently isolated from meju. However, these fungi are not the predominant species in Korean traditional meju because most typical farmers ferment meju in their houses without heating, keeping it at low temperatures (the temperature is diverse according to farmers, but it is usually less than 20°C), which is not optimum temperature for its growth. Many Jangryu factories in the central part of the Korean Peninsula, which sell meju, doenjang, and ganjang, ferment meju at higher temperatures (sometimes higher than 30°C) for 2–3 weeks. In these cases, the temperature is optimum for *A. flavus/oryzae* growth and it can actively grow. In fact, we found some meju loaves in which *A. flavus/oryzae* grew extensively, but in a significantly high proportion of meju, other fungi were already growing in the meju before *A. flavus/oryzae*.

Of the wild *A. flavus/oryzae* strains that grow in meju, only 7.3% of them can produce aflatoxin, and the fungi do not grow so actively in meju. Furthermore, all aflatoxin-producible *A. flavus/oryzae* strains do not produce aflatoxin in meju. Even if aflatoxin was produced in meju in low quantities, meju is not consumed directly, but doenjang and ganjang, which are made by fermenting meju for more than 1 year, are consumed. According to Park and Lee (1987), and Park *et al.* (1988), the aflatoxin in meju is degraded during the preparation of ganjang and doenjang. Therefore, aflatoxin contamination of doenjang and ganjang is not as severe as reported by the Time article (1969, http://www.time.com/time/magazine/article/0,9171,844829,00.html). Furthermore, there have been no reports of adverse effects of health in Korea, which were caused due to aflatoxin contamination in doenjang and ganjang. Nonetheless, much attention should be paid on the control of aflatoxin in ganjang and doenjang because aflatoxin-producible *A. flavus/oryzae* strains have been found in meju, although they exist in small proportions.

Taxonomy of the *A. flavus/oryzae* complex is ambiguous. Many mycologists have tried to differentiate between *A. oryzae* and *A. flavus* by using morphological and molecular methods (Hadrich *et al.*, 2011), but there is no clear taxonomic key for their differentiation thus far. The genome sequences of *A. oryzae* and *A. flavus* showed 99.5% similarity (Payne *et al.*, 2006; Rokas *et al.*, 2007), and mycologists generally agree that they are scientifically the same species, i.e. *A. oryzae* is a synonym of *A. flavus* because *A. flavus* was reported earlier than *A. oryzae*. However, big problems occur, if many oriental fermented foods are made by *A. flavus.* Therefore, the species, *A. oryzae* has to be maintained. Taxonomically there is no clear key to differentiate the two species, but practically the two species can be differentiated according to their aflatoxin-producing ability. All mycologists agree that *A. oryzae* cannot produce aflatoxin and that *A. flavus* does or does not produce aflatoxins. Therefore, using this criterion, Kiyota *et al.* (2011) used *A. oryzae* for defining non-aflatoxigenic *A. flavus/oryzae* strains and *A. flavus* for defining aflatoxigenic *A. flavus/oryzae* strains.

A. flavus/oryzae is common in oriental fermented foods, and *A. oryzae* is considered as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration, but *A. flavus* is regarded as a Biosafety level-2 opportunistic pathogen (de Hoog *et al.*, 2000). Therefore, it seems advantageous to name the *A. flavus/oryzae* strains from oriental foods as *A. oryzae*, only if the *A. flavus/oryzae* strain does not produce aflatoxin. Hence, we propose that species identification of *A. flavus/oryzae* from oriental foods should be based on the aflatoxin-producing capability of the strain, i.e. if the strain can produce aflatoxin, it should be identified as *A. flavus* and if the strain cannot produce aflatoxin, it should be identified as *A. oryzae*. Based on this criterion, we named 178 meju strains, which belonged to the non-aflatoxigenic group (Type I of *norB-cypA*, IB-L-B-, IC-AO, or IA-L-B- of *omtA*, and AO type of *aflR*), as *A. oryzae*, and 14 meju strains, which belonged to aflatoxin-producible group (Type II of *norB-cypA*, IC-L-B+/B- or IC-L-B+ of *omtA*, and AF type of *aflR*) as *A. flavus* in this study.

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