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Research Article

Mycoflora of Soybeans Used for Meju Fermentation

Dae-Ho Kim[1,2], Seon-Hwa Kim[1], Soon-Wo Kwon[1], Jong-Kyu Lee[2] and Seung-Beom Hong[1,*]

[1]*Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-853, Korea*
[2]*Tree Pathology and Mycology Laboratory, Division of Forest Environment Protection, Kangwon National University, Chuncheon 200-701, Korea*

Abstract Diverse fungi are present in Korean traditional meju and they are known to play an important role in fermented soybean products. To determine the origin of the fungi in meju, we examined the mycoflora of soybeans from 10 traditional meju factories. The samples were untreated or treated with sodium hypochlorite, and placed on malt extract agar (MEA), dichloran 18% glycerol agar (DG18), and dichloran rose bengal chloramphenicol agar (DRBC) medium. A total of 794 fungal strains were isolated and they were identified as 41 genera and 86 species. From sodium hypochlorite untreated soybeans, the genera, *Cladosporium* (55%), *Eurotium* (51%), *Fusarium* (33%), *Penicillium* (22%), and *Aspergillus* (exclusion of *Eurotium*) (20%), were mainly isolated, and *Eurotium herbariorum* (22%), *Eurotium repens* (18%), *Cladosporium tenuissimum* (18%), *F. fujikuroi* (18%), *Aspergillus oryzae/flavus* (7%), and *Penicillium steckii* (6%) were the predominant species. In case of sodium hypochlorite-treated soybeans, *Eurotium* (31%) and *Cladosporium* (5%) were frequently isolated, but *Aspergillus* (excluding *Eurotium*), *Penicillium* and *Fusarium* which were frequently isolated from untreated soybeans, were rarely isolated. *Eurotium herbariorum* (21%), *Eurotium repens* (8%), and *Cladosporium tenuissimum* (3%) were the predominant species. Of the 41 genera and 86 species isolated from soybeans, 13 genera and 33 species were also found in meju. These results suggest that the fungi on soybeans may influence the mycoflora of meju.

Keywords *Cladosporium tenuissimum*, *Eurotium herbariorum*, *Eurotium repens*, Fungi, Soybean

The soybean is native to the Korean Peninsula and the Manchurian area [1] and soybean has been one of the major sources of protein in Korean food [2]. Numerous foods are made by soybeans, including doenjang (soybean paste), ganjang (soybean source), cheonggukjang (fast-fermented bean paste), bean curd, soybean milk, bean-curd dregs, etc. [3]. Soybean consumption is effective for the prevention of osteoporosis, arteriosclerosis, strokes and dementia, and can reduce the risk of cancer and obesity [2]. Furthermore, fermented soybean foods are effective in preventing and curing adult diseases [3].

Korean traditional meju is an important food ingredient in Korean cuisine. Meju is made by soaking and boiling soybeans, and then ferment them with various microorganisms such as bacteria, fungi, and yeast [4]. The fungi related to meju fermentation are *Aspergillus*, *Cladosporium*, *Eurotium*, *Lichtheimia*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis*, etc. [5-7]. Mycoflora of meju could be influenced by its environmental factors such as air, rice straw and soybeans. In particular, soybeans, which are the main component of meju, are contaminated by various fungi, such as *Cercospora*, *Diaporthe*, *Colletotrichum*, *Alternaria*, *Aspergillus*, *Fusarium*, *Chaetomium*, and *Penicillium* [8]. The genera, *Aspergillus*, *Penicillium*, *Fusarium*, and *Cladosporium* have been isolated not only from soybeans but also from meju. Therefore, it seems that there is a relationship between the fungi found on soybeans and those found in meju. The aims of this study are 1) to examine the mycoflora of soybeans used for meju fermentation and 2) to compare these fungal species with those found in meju.

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*__Corresponding author__
E-mail: funguy@korea.kr

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MATERIALS AND METHODS

From November 2011 to February 2012, we collected

Table 1. Information of the soybeans used in this study

No.	Location	Cultivar
1	Chungnam, Gongju	Taekwangkong
2	Gyeongbuk, Chilgok	Unkonwn
3	Gyeonggi, Ansung	Daewonkong
4	Gyeonggi, Icheon	Daewonkong
5	Gyeonggi, Yangpyeong	Taekwangkong
6	Gyeonggi, Yongin	Taekwangkong
7	Jeonbuk, Sunchang D	Taekwangkong
8	Jeonbuk, Sunchang H	Taekwangkong
9	Jeonnam, Gangjin	Taekwangkong
10	Jeonnam, Haenam	Taekwangkong

soybeans used for meju from 10 traditional meju factories. The information of collected soybeans is listed in Table 1.

To elucidate whether fungi exist on the surface or inside of soybeans, soybean samples were either untreated or treated with sodium hypochlorite (0.5%) for 1 min. Sodium hypochlorite treated soybeans were washed with distilled water then dried. Fifty kernels were placed (5 kernels per plate) on malt extract agar (MEA), dichloran rose bengal chloramphenicol agar (DRBC), and dichloran 18% glycerol agar (DG18) [9], respectively. After 5~7 days of incubation at 25°C in the dark, fungi growing on the soybeans and media were transferred to new MEA or DG18 (for xerophilic fungi). The grown fungi were examined with a light microscope and were maintained at 4°C after transferring to MEA or DG18 slant.

Molecular and morphological methods were used to identify the fungi. To extract genomic DNA, all strains were cultured in malt extract broth or DG18 (for xerophilic fungi). After harvesting the mycelium, genomic DNA was extracted using the DNeasy Plant Mini Kit (69106; Qiagen, Hilden, Germany) according to the manufacturer's instructions, and then stored at −20°C until use. For identification, several genes were analyzed depending on the genus: the partial sequence of the beta-tubulin gene [10] for *Aspergillus*, *Eurotium* and *Penicillium*; the partial sequence of the actin gene [11] for *Cladosporium*; the partial sequence of the elongation factor 1-alpha gene [12] for *Fusarium*, and an internal transcribed spacer of nuclear ribosomal DNA [13] for the other genera. Morphological characteristics of all strains were analyzed according to the methods of Samson *et al.* [14] and Pitt and Hocking [15].

RESULTS AND DISCUSSION

A total of 794 fungal strains were isolated from 93% of sodium hypochlorite untreated soybeans and from 37% of sodium hypochlorite-treated soybeans, and were identified as 41 genera and 86 species (Table 2). There was no correlation between mycoflora with the region of collection or the soybean cultivar (*Daewonkong* and *Taekwangkong*).

The occurrence and frequency of fungi differed according to the media. Most fungi grew well on DRBC, and Pitt and Hocking [16] suggested that DRBC is adequate for the numeration of fungi on food and feed. In this study, the number of isolated fungi and the incidence on soybeans were based on fungi grown on DRBC. However, *Eurotium* did not grow well on DRBC, but did grow well on DG18. Therefore, the number and incidence of *Eurotium* were from fungi grown on DG18, which is medium for xerophilic fungi [9].

In the case of untreated soybeans, 32 genera and 68 species were isolated (Table 2). *Cladosporium* was isolated from 273 of 500 soybeans (54.6%), and from 9 of 10 factories. *Eurotium* was isolated from 254 of 500 soybeans (50.8%) and from 7 of 10 factories. *Fusarium*, *Penicillium*, and *Aspergillus* (excluding *Eurotium*) were isolated from all 10 factories and their incidences on soybeans were 32.8%, 22.4%, and 20%, respectively. All other genera were isolated from less than 4% of soybeans. Although *Alternaria* (3.4%), *Rhizopus* (3%), *Phoma* (2.8%), *Phomopsis* (1%), *Cheatomium* (1%), and *Epicoccum* (1%) occurred infrequently, they were isolated from several factories. *Lichtheimia* (3.8%) and *Monascus* (0.6%), each were isolated from 2 factories. All other genera were isolated from only 1 factory. *Eurotium herbariorum* (22.4%), *E. repens* (18.4%), *F. fujikuroi* (anamorph of *Gibberella fujikuroi*) (18.4%), and *Cladosporium tenuissimum* (18.2%) were major species isolated from untreated soybeans, followed by *C. pseudocladosporioides* (9.4%), *C. cladosporioides* (9%), *Eurotium manginii* (8.4%), *Paecilomyces* sp. (7.6%), *A. oryzae/flavus* (7%), *A. westerdijkiae* (6.2%), and *A. versicolor* (5.4%).

A total of 28 genera and 52 species were isolated from the sodium hypochlorite-treated soybeans (Table 2). Among these, the most frequent genus, *Eurotium*, was isolated from 153 of 500 soybeans (30.6%) on DG18, and from 9 of 10 factories. The second most frequent genus was *Cladosporium*, which was isolated from 27 of 500 soybeans (5.4%) on DRBC, and from 7 of 10 factories. The next frequent fungi were *Fusarium* (from 8 factories) and *Aspergillus* (excluding *Eurotium*) (8), which were isolated from 2.2% and 2% of soybeans, respectively, followed by *Cercospora* (3) and *Botryosphaeria* (3). Other genera, such as *Alternaria*, *Chaetomium*, *Penicillium*, *Phoma*, and *Phomopsis*, were isolated from less than 1% of soybeans, but were isolated from quite a few factories. *E. herbariorum* (21.2%), *E. repens* (8%), and *C. tenuissimum* (2.6%) were the major species isolated from sodium hypochlorite-treated soybeans, followed by *Cercospora* sp. (1.2%), *B. dothidea* (1%), and *C. pseudocladosporioides* (1%). Other species were isolated from less than 1% of soybeans.

Fourteen species of *Aspergillus* (excluding *Eurotium*) were isolated from untreated soybeans, and *A. oryzae/flavus*, *A. westerdijkiae*, and *A. versicolor* were the major species isolated from soybeans (Table 2). Of these, *A. oryzae/flavus* is one of the major fungi for meju fermentation, whereas *A. westerdijkiae* and *A. versicolor* are considered simple contaminants of meju that are found very infrequently (unpublished data). *Eurotium*, the teleomorph of *Aspergillus*

Table 2. List of fungal species from soybeans and their isolation frequencies

Scientific name	Representative strains		Incidence on meju[b]	Isolation frequency from sodium hypochlorite untreated soybeans[c]				Isolation frequency from sodium hypochlorite untreated soybeans[c]			
	Strain No.	Sequence No.[a]		No. of factories[d]	DRBC	DG18	MEA	No. of factories[d]	DRBC	DG18	MEA
Acremonium				1	1	-	-	-	-	-	-
Acremonium sp.	KACC 47123	RDA0041296		1	1	-	-	-	-	-	-
Alternaria				7	17	1	8	6	4	5	7
Alternaria sp.	KACC 47124	RDA0041297		7	17	1	8	7	4	5	7
Arthrinium				1	19	7	5	2	3	1	1
A. phaeospermum	KACC 47125	RDA0041298	*	1	17	7	4	2	3	1	1
Arthrinium sp.	KACC 47126	RDA0041299		1	5	-	1	-	-	-	-
Aspergillus				10	100	101	47	8	10	11	6
A. aculeatus	KACC 47127	RDA0041333		1	-	-	1	-	-	-	-
A. creber	KACC 47128	RDA0041334		-	-	-	-	1	2	-	-
A. fumigatus	KACC 47129	RDA0041335	**	1	1	-	-	-	-	-	-
A. jensenii	KACC 47130	RDA0041336		2	-	1	1	1	-	-	1
A. malodoratus	KACC 47131	RDA0041337		1	1	-	-	-	-	-	-
A. nidulans	KACC 47140	RDA0041345	**	1	-	2	-	-	-	-	-
A. ochraceus	KACC 47132	RDA0041338	**	4	3	13	5	1	1	-	1
A. oryzae/flavus	KACC 47133	RDA0041381	***	7	35	36	18	1	1	-	1
A. ostianus	KACC 47134	RDA0041339		1	-	1	-	-	-	-	-
A. steynii/elegans	KACC 47135	RDA0041340		1	1	-	1	-	-	-	-
A. sydowii	KACC 47136	RDA0041341	**	4	-	18	-	-	-	-	-
A. tubingensis	KACC 47137	RDA0041342	**	2	3	2	1	2	1	1	-
A. versicolor	KACC 47138	RDA0041343	**	4	27	10	4	3	3	2	1
A. westerdijkiae	KACC 47139	RDA0041344	*	3	31	15	13	1	-	-	2
Aspergillus sp.				5	17	9	6	3	1	4	-
Bionectria				1	1	-	1	-	-	-	-
B. epichloe	KACC 47151	RDA0041300		1	1	-	1	-	-	-	-
Biscogniauxia				-	-	-	-	1	1	-	-
B. mediterranea	KACC 47152	RDA0041301		-	-	-	-	1	1	-	-
Botryosphaeria				1	-	1	-	3	5	1	3
B. dothidea	KACC 47153	RDA0041302		1	-	1	-	3	5	1	3
Botrytis			*	1	1	-	1	-	-	-	-
Botrytis sp.	KACC 47154			1	1	-	1	-	-	-	-
Cercospora				-	-	-	-	3	6	1	2
Cercospora sp.	KACC 47155	RDA0041303		-	-	-	-	3	6	1	2
Chaetomium			*	4	5	-	2	3	1	1	1
C. globosum	KACC 47156	RDA0041304		2	3	-	-	-	-	-	-
Chaetomium sp.	KACC 47157	RDA0041305		2	2	-	2	3	1	1	1

Table 2. Continued

Scientific name	Representative strains		Incidence on meju[b]	Isolation frequency from sodium hypochlorite untreated soybeans[c]				Isolation frequency from sodium hypochlorite untreated soybeans[c]			
	Strain No.	Sequence No.[a]		No. of factories[d]	DRBC	DG18	MEA	No. of factories[d]	DRBC	DG18	MEA
Cladosporium				7	273	189	98	7	27	51	22
C. cladosporioides	KACC 47158	RDA0041369	**	1	45	-	-	1	1	9	-
C. halotolerans	KACC 47159	RDA0041370		-	-	-	-	1	1	-	-
C. pseudocladosporioides	KACC 47160	RDA0041371		1	47	-	6	1	5	17	8
C. sphaerospermum	KACC 47161	RDA0041372	*	1	-	1	-	-	-	-	-
C. tenuissimum	KACC 47162	RDA0041373	**	2	91	97	35	3	13	22	11
Cladosporium sp.				4	138	92	57	2	7	3	3
Cochliobolus				-	-	-	-	3	1	8	3
Cochliobolus sp.	KACC 47163	RDA0041306		-	-	-	-	3	1	8	3
Colletotrichum				1	-	-	1	2	3	-	1
Colletotrichum sp.	KACC 47164	RDA0041307		1	-	-	1	2	3	-	-
Corynespora				1	1	-	-	-	-	-	-
C. cassiicola	KACC 47165	RDA0041308		1	1	-	-	-	-	-	-
Epicoccum				3	5	1	-	1	-	-	1
E. nigrum	KACC 47166	RDA0041309	**	1	1	-	-	1	-	-	1
Epicoccum sp.	KACC 47167	RDA0041310		2	4	1	-				
Eurotium (*Aspergillus* section *Aspergillus*)				9	(39)	254	(15)	9	(38)	153	(24)
Aspergillus cibarius	KACC 47141	RDA0041346	*	-	-	-	-	1	(1)	4	-
E. amstelodami	KACC 47142	RDA0041347	**	1	-	1	-	-	-	-	-
E. chevalieri	KACC 47143	RDA0041348	***	3	-	16	-	-	-	-	-
E. echinulatum	KACC 47144	RDA0041349	**	3	-	4	-	-	-	-	-
E. herbariorum	KACC 47145	RDA0041350	**	8	-	112	-	6	(14)	106	(1)
E. manginii	KACC 47146	RDA0041351		2	-	42	-	1	(1)	7	(2)
E. niveoglaucum/medium	KACC 47147	RDA0041352		2	-	6	-	1	-	1	-
E. repens	KACC 47148	RDA0041353	***	6	(6)	92	-	7	(29)	40	(20)
E. rubrum	KACC 47149	RDA0041354	**	3	-	15	-	1	(1)	4	(1)
E. tonophilum	KACC 47150	RDA0041355	*	1	-	1	-	2	-	18	-
Eurotium sp.				3	(33)	19	(15)	1	-	2	-
Fusarium				10	164	45	141	8	11	16	4
F. andiyazi	KACC 47168	RDA0041374		-	-	-	-	1	1	-	-
F. concentricum	KACC 47169	RDA0041375		2	1	1	-	1	-	1	1
F. fujikuroi (anamorph of *Gibberella fujikuroi*)	KACC 47170	RDA0041376	*	5	92	14	97	3	3	1	1
F. cf. *incarnatum*	KACC 47171	RDA0041377	*	2	2	9	1	2	3	2	2
F. oxysporum	KACC 47172	RDA0041378		2	1	-	1	2	1	1	-
F. proliferatum	KACC 47173	RDA0041379		2	7	15	4	-	-	-	-
F. solani	KACC 47174	RDA0041380		1	5	3	2	1	1	-	-
Fusarium sp.				5	61	4	37	3	3	11	-

Table 2. Continued

Scientific name	Representative strains		Incidence on meju[b]	Isolation frequency from sodium hypochlorite untreated soybeans[c]				Isolation frequency from sodium hypochlorite untreated soybeans[c]			
	Strain No.	Sequence No.[a]		No. of factories[d]	DRBC	DG18	MEA	No. of factories[d]	DRBC	DG18	MEA
Irpex				1	4	3	1	-	-	-	-
I. lacteus	KACC 47175	RDA0041311		1	4	3	1	-	-	-	-
Khuskia				1	2	-	6	1	-	-	3
K. oryzae	KACC 47176	RDA0041312		-	-	-	-	1	-	-	3
Khuskia sp.	KACC 47177	RDA0041313		1	2	-	6	-	-	-	-
Leptosphaerulina				-	-	-	-	1	-	-	1
Leptosphaerulina sp.	KACC 47178	RDA0041314		-	-	-	-	1	-	-	1
Lichtheimia				2	19	3	16	-	-	-	-
L. corymbifera	KACC 47179	RDA0041315	**	1	-	-	1	-	-	-	-
L. ramosa	KACC 47180	RDA0041316	***	2	19	3	15	-	-	-	-
Microascus				-	-	-	-	1	-	-	1
Microascus sp.	KACC 47181			-	-	-	-	1	-	-	1
Monascus			**	2	3	-	2	1	-	-	1
Monascus sp.	KACC 47182			2	3	-	2	1	-	-	1
Monographella				1	1	-	-	1	1	-	-
Monographella sp.	KACC 47183	RDA0041317		1	1	-	-	1	1	-	-
Mucor				1	-	-	1	-	-	-	-
Mucor sp.	KACC 47184			1	-	-	1	-	-	-	-
Nigrospora				-	-	-	-	1	-	2	-
N. oryzae	KACC 47185	RDA0041318		-	-	-	-	1	-	2	-
Neurospora				1	13	1	11	1	1	-	-
Neurospora sp.	KACC 47186			1	13	1	11	1	1	-	-
Paecilomyces				1	38	-	19	1	1	-	1
Paecilomyces sp.	KACC 47187			1	38	-	19	1	1	-	1
Penicillium				10	112	54	51	6	3	25	2
P. cecidicola	KACC 47188	RDA0041356		1	2	-	-	-	-	-	-
P. chrysogenum complex	KACC 47189	RDA0041357	**	2	6	4	1	-	-	-	-
P. citrinum	KACC 47190	RDA0041358		1	-	7	-	1	-	1	-
P. cyclopium	KACC 47191	RDA0041359	*	-	-	-	-	1	-	-	2
P. expansum	KACC 47192	RDA0041360	*	2	3	-	2	-	-	-	-
P. ochrochloron	KACC 47193	RDA0041361		1	1	-	-	-	-	-	-
P. oxalicum	KACC 47194	RDA0041362	*	1	20	7	5	1	-	1	-
P. polonicum	KACC 47195	RDA0041363	***	4	21	12	4	-	-	-	-
P. roqueforti	KACC 47196	RDA0041364	**	1	1	-	-	-	-	-	-
P. rubrum/purpurogenum	KACC 47197	RDA0041365		2	1	-	2	1	-	1	-
P. steckii	KACC 47198	RDA0041366	*	5	32	12	22	1	1	-	-
P. sumatrense	KACC 47199	RDA0041367		1	-	5	-	-	-	-	-
P. toxicarium	KACC 47200	RDA0041368		-	-	-	-	1	1	-	-
Penicillium sp.				6	28	9	17	2	1	22	-

Table 2. Continued

Scientific name	Representative strains		Incidence on meju[b]	Isolation frequency from sodium hypochlorite untreated soybeans[c]				Isolation frequency from sodium hypochlorite untreated soybeans[c]			
	Strain No.	Sequence No.[a]		No. of factories[d]	DRBC	DG18	MEA	No. of factories[d]	DRBC	DG18	MEA
Pestalotiopsis				1	-	13	-	1	-	1	1
Pestalotiopsis sp.	KACC 47201	RDA0041319		1	-	13	-	1	-	1	1
Phoma			*	5	14	1	8	3	3	-	1
P. herbarum	KACC 47202	RDA0041320		3	8	-	5	1	1	-	-
Phoma sp.	KACC 47203	RDA0041321		5	7	1	3	2	2	-	1
Phomopsis				4	5	-	17	4	2	1	3
P. longicolla	KACC 47204	RDA0041322						1	-	-	1
Phomopsis sp.	KACC 47205	RDA0041323		4	5	-	15	3	2	1	2
Rhizopus				3	15	9	15	-	-	-	-
R. microsporus	KACC 47206	RDA0041324		1	1	-	-	-	-	-	-
Rhizopus sp.	KACC 47207			2	14	9	15	-	-	-	-
Schizophyllum				1	1	-	-	-	-	-	-
S. commune	KACC 47208	RDA0041325		1	1	-	-	-	-	-	-
Scopulariopsis				-	-	-	-	1	1	2	1
S. chartarum/trigonospora	KACC 47209	RDA0041326		-	-	-	-	1	1	2	1
Sphaerodes				-	-	-	-	1	1	-	-
Sphaerodes sp.	KACC 47210	RDA0041327		-	-	-	-	1	1	-	-
Stemphylium				-	-	-	-	1	-	-	2
Stemphylium sp.	KACC 47211	RDA0041328		-	-	-	-	1	-	-	2
Syncephalastrum				1	-	-	1	-	-	-	-
S. racemosum	KACC 47212	RDA0041329	*	1	-	-	1	-	-	-	-
Talaromyces				1	-	-	2	-	-	-	-
Talaromyces sp.	KACC 47213	RDA0041330		1	-	-	2	-	-	-	-
Trhicoderma				1	1	-	-	-	-	-	-
Trhicoderma sp.	KACC 47214			1	1	-	-	-	-	-	-
Trichothecium				1	3	-	-	-	-	-	-
T. roseum	KACC 47215	RDA0041331		1	3	-	-	-	-	-	-
Verticillium				-	-	-	-	1	1	-	-
V. nigrescens	KACC 47216	RDA0041332		-	-	-	-	1	1	-	-

[a]The RDA numbers are DNA sequence accession number of Korean Agricultural Culture Collection (KACC). Readers can access to the sequence from information of corresponding KACC No. in KACC homepage (http://www.genebank.go.kr).
[b]The species were isolated from meju, with ***high frequency, **medium frequency, or *low frequency.
[c]The frequency indicates the number of soybeans from which the species were isolated from 500 soybeans.
[d]The number indicates the number of factories from which the species were isolated from 10 factories.

section *Aspergillus*, were frequently isolated from both sodium hypochlorite untreated and treated soybeans on DG18 (Table 2). *E. herbariorum*, *E. repens* and *E. manginii* were the major species. From meju, *E. repens* and *E. chevalieri* were the predominant species [5]. Five species of *Cladosporium* were isolated from both untreated and sodium hypochlorite-treated soybeans on DRBC (Table 2). The *Cladosporium* spp. from soybeans were not isolated from many factories, but although these species were found on soybeans from a factory, the incidence rate was high. *Cladosporium* sp., *C. tenuissimum*, *C. pseudocladosporioides*, and *C. cladosporioides* were mainly isolated from soybeans, whereas *C. tenuissimum* and *C. sphaerospermum* were predominant in meju (unpublished data). In case of *Fusarium*, *F. fujikuroi* (anamorph of *Gibberella fujikuroi*) and *Fusarium* sp. were frequently isolated from untreated soybeans (92 and 61 out of 500 soybeans, respectively) on DRBC, however they were rarely isolated from sodium hypochlorite-treated soybeans (Table 2). This means that the species mainly exist on the surface of soybeans and are not pathogens of soybeans. In meju, *Fusarium asiaticum* is a predominant species (unpublished data). Although 13 species of *Penicillium* were isolated from soybeans, and *P. steckii*, *Penicillium* sp., *P. polonicum*, and *P. oxalicum* were the major species, almost of them were isolated from untreated soybeans, and the incidence rates were not high (Table 2). In case of *P. polonicum*, it is also frequently isolated from meju [4]. Meju is a nutrient rich material for zygomycota, and *Mucor*, *Lichtheimia*, and *Rhizopus* grow well on/in it [6]. However, soybeans are dry, and zygomycota cannot utilize its nutrients. Therefore, zygomycota were rarely isolated from untreated soybeans on MEA (*Lichtheimia*, 16 of 500 soybeans; *Mucor*, 1 of 500; and *Rhizopus*, 15 of 500) and were not isolated from sodium hypochlorite-treated soybeans. Among the other genera, *Paecilomyces*, *Alternaria*, *Phomopsis*, and *Phoma* were quite frequently isolated from untreated soybeans, and *Alternaria*, *Cochliobolus*, *Botryosphaeria*, and *Cercospora*, which could be soybean pathogens, were isolated from sodium hypochlorite-treated soybeans.

The fungi isolated from sodium hypochlorite-treated soybeans may be endophytic or pathogenic fungi. *Fusarium oxysporum* and *F. solani* were reported as pathogens causing Fusarium wilt, Fusarium blight and root rot of soybeans in Korea [17]. *Phomopsis longicolla* and *Corynespora cassiicola* were reported as pathogenic fungi to soybeans in other countries [18, 19]. Additionally, *B. dothidea*, *F. fujikuroi*, *Khuskia oryzae*, and *P. oxalicum*, were reported as pathogenic fungi in other plants [17]. Therefore, fungi isolated from sodium hypochlorite-treated soybeans may have a potential to cause disease or affect the growth of soybeans. Research about the role of fungi on soybeans will be necessary.

Yum and Park [8] isolated 16 fungal genera from sodium hypochlorite-treated yellow soybeans collected from factories or markets in various regions of Korea, and *Cercospora*, *Diaporthe*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Colletotrichum*, *Penicillium*, and *Cladosporium* were frequently isolated. Of these genera, *Alternaria*, *Aspergillus*, *Cercospora*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, and *Penicillium* were also isolated in this study. However, *Diaporthe* was not isolated in this study. In contrast, 19 genera, including *Eurotium*, were only isolated in this study. Pitt *et al.* [20] isolated 26 genera and 59 species from 49 samples of surface-disinfected soybeans collected from markets in Thailand. *Penicillium restrictum*, *Aspergillus penicilloides*, *A. restrictus*, *Cladosporium cladosporioides*, *Eupenicillium cinnamopurpureum*, *Eurotium amstelodami*, *Eur. chevalieri*, *Eur. Rubrum*, and *P. citrinum* were frequently isolated. Among the 26 genera from the study by Pitt *et al.* [20], 12 genera, including *Alternaria*, *Arthrinium*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Epicoccum*, *Eurotium*, *Fusarium*, *Penicillium*, *Pestalotiopsis*, and *Phoma*, were also isolated in the present study. However, 14 genera, including *Curvularia*, *Eupenicillium*, *Lasiodiplodia*, *Macrophomina*, *Nigrospora*, *Rhizopus*, and *Syncephalastrum*, were not isolated in the present study. In contrast, 16 genera, including *Botryosphaeria*, *Cercospora*, *Cochliobolus*, *Khuskia*, *Phomopsis*, and *Stemphylium*, were isolated in the present study, but were not isolated in the study by Pitt *et al.* [20]. The mycoflora isolated in this study were more similar to that of Pitt *et al.* [20] than that of Yum and Park [8], even though the former used Thai soybeans and the latter used Korean soybeans. In particular, *Eurotium* was one of the most frequently isolated fungi in the present study and in Pitt *et al.* [20] however, Yum and Park [8] did not isolate the genus. This is likely caused by the use of different media. Yum and Park [8] only used potato sucrose agar containing streptomycin sulfate in which *Eurotium* rarely grow, whereas Pitt *et al.* [20] used DG18, DRBC, dichloran chloramphenicol peptone agar, and *Aspergillus flavus* and *parasiticus* agar.

Among the 41 genera and 86 species isolated from soybeans, 13 genera and 33 species were isolated from both soybeans and meju. The fungi on soybeans cannot be directly transferred into meju, because soybeans are sterilized during the boiling process during which producers usually boil the soybeans in water for more than 4 hr. Only

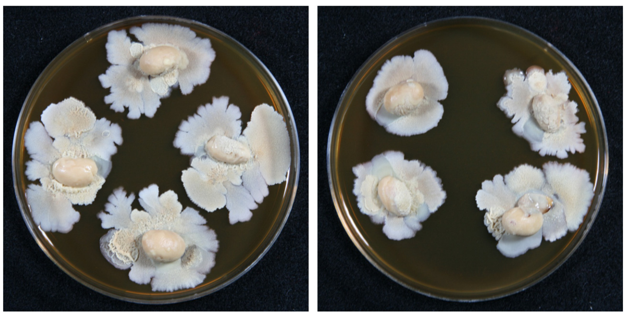

Fig. 1. The incubation results of boiled soybeans on MEA after 7 days at 25°C in the dark. The soybeans were boiled in an iron pot for 6 hr in a meju factory in Icheon, Korea. Fungi could not grow and only *Bacillus* strains could grow.

Bacillus could survive this step (Fig. 1). However, the fungi on the soybeans may move into the air during the washing process, and may be re-inoculated on the meju during or after meju forming process. This study suggests that some fungi on soybeans, such as *Aspergillus*, *Eurotium*, and *Penicillium*, may influence the mycoflora of meju fermentation.

Selected strains from this study have been preserved in the Korean Agricultural Culture Collection (KACC; http://www.genebank.go.kr) and are accessible for future research.

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