

# The Mycobiota of Air Inside and Outside the Meju Fermentation Room and the Origin of Meju Fungi

Dae-Ho Kim<sup>1,2</sup>, Sun-Hwa Kim<sup>1</sup>, Soon-wo Kwon<sup>1</sup>, Jong-Kyu Lee<sup>2</sup> and Seung-Beom Hong<sup>1,\*</sup>

<sup>1</sup>Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Wanju 55365, Korea

<sup>2</sup>Tree Pathology and Mycology Laboratory, Division of Forest Environment Protection, Kangwon National University, Chuncheon 24341, Korea

**Abstract** The fungi on Meju are known to play an important role as degrader of macromolecule of soybeans. In order to elucidate the origin of fungi on traditional Meju, mycobiota of the air both inside and outside traditional Meju fermentation rooms was examined. From 11 samples of air collected from inside and outside of 7 Meju fermentation rooms, 37 genera and 90 species of fungi were identified. In outside air of the fermentation room, *Cladosporium* sp. and *Cladosporium cladosporioides* were the dominant species, followed by *Cladosporium tenuissimum*, *Eurotium* sp., *Phoma* sp., *Sistotrema brinkmannii*, *Alternaria* sp., *Aspergillus fumigatus*, *Schizophyllum commune*, and *Penicillium glabrum*. In inside air of the fermentation room, *Cladosporium* sp., *Aspergillus oryzae*, *Penicillium chrysogenum*, *Asp. nidulans*, *Aspergillus* sp., *Cl. cladosporioides*, *Eurotium* sp., *Penicillium* sp., *Cl. tenuissimum*, *Asp. niger*, *Eur. herbariorum*, *Asp. sydowii*, and *Eur. repens* were collected with high frequency. The concentrations of the genera *Aspergillus*, *Eurotium*, and *Penicillium* were significantly higher in inside air than outside air. From this result and those of previous reports, the origin of fungi present on Meju was inferred. Of the dominant fungal species present on Meju, *Lichtheimia ramosa*, *Mucor circinelloides*, *Mucor racemosus*, and *Scopulariopsis brevicaulis* are thought to be originated from outside air, because these species are not or are rarely isolated from rice straw and soybean; however, they were detected outside air of fermentation room and are species commonly found in indoor environments. However, *Asp. oryzae*, *Pen. polonicum*, *Eur. repens*, *Pen. solitum*, and *Eur. chevalieri*, which are frequently found on Meju, are common in rice straw and could be transferred from rice straw to Meju. The fungi grow and produce abundant spores during Meju fermentation, and after the spores accumulate in the air of fermentation room, they could influence mycobiota of Meju fermentation in the following year. This could explain why concentrations of the genera *Aspergillus*, *Eurotium*, and *Penicillium* are much higher inside than outside of the fermentation rooms.

**Keywords** Air, Fungi, Meju, Mycobiota, Origin

Meju is the important raw material for traditional Korean Jangryu (the singular form, Jang) such as Ganjang, Doenjang, and Gochujang [1]. Jangryu are useful and important sauces

in Korean cuisine. Moreover, Jangryu have been reported to have health benefits such as antioxidative activity, antithrombotic effects, cholesterol-lowering effects, mutation suppression effects, and antitumor activities [2]. The taste and quality of Jangryu are decided by the Meju used to make them [3]. Various microorganisms are associated with Meju fermentation, because traditional Meju is naturally fermented [3]. In particular, fungi, which produce various enzymes and degrade macromolecules in soybeans, are important microorganisms in Meju fermentation [3, 4]. The fungi *Aspergillus oryzae*, *Eurotium chevalieri*, *E. repens*, *Lichtheimia ramosa*, *Mucor circinelloides*, *M. racemosus*, *Penicillium polonicum*, *P. solitum*, and *Scopulariopsis brevicaulis* occur commonly on Meju [4-8].

In this study, we investigated the origins of the fungi on traditional Meju fermentation. This knowledge would help to control of fungi that are present during Meju fermentation, as some fungi on Meju are helpful for fermentation, but

Mycobiology 2015 September, 43(3): 258-265  
<http://dx.doi.org/10.5941/MYCO.2015.43.3.258>  
pISSN 1229-8093 • eISSN 2092-9323  
© The Korean Society of Mycology

**\*Corresponding author**

E-mail: funguy@korea.kr

**Received** August 18, 2015

**Revised** August 31, 2015

**Accepted** September 6, 2015

©This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

others are simply contaminants or toxigenic [2, 9]. Possible sources of the fungi on traditional *Meju* include rice straw, soybeans, and the air inside and outside the fermentation room. The fungi present on rice straw and soybean have been previously reported [10, 11]. Therefore, the aims of this study are to (1) identify the composition of the mycobiota in the air both inside and outside *Meju* fermentation room; (2) compare it with those of traditional *Meju*, rice straw, and soybeans; and (3) presume the origin of the fungi on traditional *Meju*.

## MATERIALS AND METHODS

Eleven air samples were collected from inside (the fermentation room) and outside (surrounding *Jang* factory) of 7 *Meju* fermentation rooms during November 2011, before starting *Meju* production (Table 1). The samples were collected using a MAS-100 Eco air sampler (Merck, Darmstadt, Germany) with malt extract agar (MEA; for general fungi), dichloran rose bengal chloramphenicol agar (for general enumeration of fungi), dichloran 18% glycerol agar (DG18; for xerophilic fungi), and tryptic soy agar (for

the genus *Scopulariopsis*). The air sampler put on the central floor of fermentation room or on the ground outside the *Jang* factory. And, 50 L, 100 L, and 500 L of air were collected from inside of fermentation room and 100 L, 500 L, and 1,000 L of air were collected from the outside of *Jang* factory. The plates were incubated at 25°C in the dark for 3~5 days. The colonies grown on each plate were counted, and the average fungal concentrations of each plate were expressed as colony forming units per cubic meter (CFU/m<sup>3</sup>). The fungi grown on media were transferred into new MEA or DG18 media. After incubation for several days, the fungi were examined by light microscope for simple identification, transferred into MEA or DG18 slant for further examination, and then maintained at 4°C.

Molecular and morphological characteristics were used to identify the fungi via methods previously described in Kim *et al.* [11].

## RESULTS AND DISCUSSION

As determined from 11 air samples from 7 *Jang* factories, the average concentrations of fungi inside and outside

**Table 1.** Information about and fungal concentrations of air samples from *Jang* factories

<i>Jang</i> factories			Sampling date	Total fungal concentrations in outside air (CFU/m <sup>3</sup> )	Total fungal concentrations in inside air (CFU/m <sup>3</sup> )
Label	Geological location	Fermenting room type			
GBC	Gyeongbuk, Chilgok	Room (farm village)	1 Dec 2011	201	2,611
GGI	Gyeonggi, Icheon	Room (farm village)	28 Nov 2011	401	1,425
GGY	Gyeonggi, Yongin	Greenhouse (farm village)	28 Nov 2011	686	-
JBB	Jeonbuk, Buan	Room (fishing village)	29 Nov 2011	50	1,322
JBSd	Jeonbuk, Sunchang	Room (town)	30 Nov 2011	-	1,358
JBSh	Jeonbuk, Sunchang	Yard (town)	30 Nov 2011	1,699	-
JND	Jeonnam, Damyang	Storage (farm village)	29 Nov 2011	1,380	902

**Table 2.** List of fungal species from air of *Meju* fermentation room with their concentration in air

Fungal species	KACC No.	Sequence No. <sup>a</sup>	Outside air		Inside air	
			No. of factories <sup>b</sup>	Total concentrations <sup>c</sup> (CFU/m <sup>3</sup> )	No. of factories <sup>b</sup>	Total concentrations <sup>c</sup> (CFU/m <sup>3</sup> )
<i>Acremonium</i>			1	10	-	-
<i>A. implicatum</i>	47372	RDA0043336	1	10	-	-
<i>Alternaria</i>			3	112	2	72
<i>Alternaria</i> sp.	47373	RDA0043364	3	112	2	72
<i>Arthriniium</i>			3	52	2	12
<i>A. sacchari</i>	47374	RDA0043363	3	42	2	12
<i>Arthriniium</i> sp.	47375	RDA0043362	1	10	-	-
<i>Aspergillus</i>			4	156	5	3,274
<i>A. caesiellus</i>	47377	RDA0043360	-	-	1	20
<i>A. creber</i>	47378	RDA0043359	-	-	1	2
<i>A. fumigatus</i>	47379	RDA0043358	2	112	1	2
<i>A. jensenii</i>	47380	RDA0043357	-	-	2	30
<i>A. nidulans</i>	47382	RDA0043355	-	-	1	450
<i>A. niger</i>	47383	RDA0043354	1	3	4	292
<i>A. ochlanceus</i>	47384	RDA0043353	-	-	1	20
<i>A. oryzae</i>	47385	RDA0043352	2	3	4	1,396
<i>A. restrictus</i>	47386	RDA0043351	-	-	1	10
<i>A. sclerotiorum</i>	47387	RDA0043350	1	22	2	70

Table 2. Continued

Fungal species	KACC No.	Sequence No. <sup>a</sup>	Outside air		Inside air	
			No. of factories <sup>b</sup>	Total concentrations <sup>c</sup> (CFU/m <sup>3</sup> )	No. of factories <sup>b</sup>	Total concentrations <sup>c</sup> (CFU/m <sup>3</sup> )
<i>A. steynii</i>	47388	RDA0043349	-	-	2	130
<i>A. sydowii</i>	47389	RDA0043348	1	2	3	250
<i>A. tubingensis</i>	47391	RDA0043346	2	3	1	10
<i>A. westerdijkiae</i>	47394	RDA0043343	1	11	-	-
<i>A. versicolor</i>	47392	RDA0043345	-	-	2	160
<i>Aspergillus</i> sp.	47393	RDA0043344	-	-	3	432
<i>Beauveria</i>			1	10	3	50
<i>B. bassiana</i>	47395	RDA0043342	1	10	3	50
<i>Bionectria</i>			1	2	1	12
<i>B. ochroleuca</i>	47396	RDA0043341	1	2	1	2
<i>Bionectria</i> sp.	47397	RDA0043340	-	-	1	10
<i>Bjerkandera</i>			2	20	3	130
<i>B. adusta</i>	47398	RDA0043339	2	20	3	130
<i>Ceriporia</i>			2	11	-	-
<i>C. lacerata</i>	47399	RDA0043338	2	11	-	-
<i>Chaetomium</i>			1	40	2	22
<i>C. globosum</i>	47400	RDA0043337	1	40	2	22
<i>Cladosporium</i>			5	3,426	5	3,000
<i>C. cladosporioides</i>	47402	RDA0043391	1	1,360	2	380
<i>C. pseudocladosporioides</i>	47403	RDA0043390	1	8	-	-
<i>C. tenuissimum</i>	47405	RDA0043388	1	490	1	310
<i>Cladosporium</i> sp.	47404	RDA0043389	5	1,568	5	2,310
<i>Cochliobolus</i>			1	1	2	12
<i>C. miyabeanus</i>	47406	RDA0043387	1	1	1	10
<i>Cochliobolus</i> sp.	47407	RDA0043386	-	-	1	2
<i>Coprinellus</i>			-	-	1	20
<i>C. radians</i>	47408	RDA0043385	-	-	1	10
<i>Coprinellus</i> sp.	47409	RDA0043384	-	-	1	10
<i>Curvularia</i>			1	1	-	-
<i>C. intermedia</i>	47410	RDA0043383	1	1	-	-
<i>Epicoccum</i>			4	31	2	32
<i>E. nigrum</i>	47412	RDA0043381	3	26	2	12
<i>Epicoccum</i> sp.	47413	RDA0043380	1	5	1	20
<i>Eurotium</i>			5	411	5	850
<i>E. chevalieri</i>	47414	RDA0043379	1	30	1	10
<i>E. herbariorum</i>	47415	RDA0043378	2	54	2	280
<i>E. repens</i>	47417	RDA0043376	2	18	3	200
<i>Eurotium</i> sp.			4	309	5	360
<i>Fusarium</i>			5	84	4	120
<i>F. acuminatum</i>	47419	RDA0043374	-	-	1	20
<i>F. asiaticum</i>	47420	RDA0043373	4	43	2	80
<i>F. proliferatum</i>	47422	RDA0043371	1	10	1	20
<i>Fusarium</i> sp.	47421	RDA0043372	2	31	-	-
<i>Irpex</i>			-	-	1	20
<i>I. lacteus</i>	47423	RDA0043370	-	-	1	20
<i>Isaria</i>			-	-	1	20
<i>I. fumosorosea</i>	47424	RDA0043369	-	-	1	20
<i>Lecanicillium</i>			1	10	2	30
<i>L. psalliotae</i>	47425	RDA0043368	1	10	2	30
<i>Lichtheimia</i>			-	-	1	2
<i>Lichtheimia</i> sp.	47426	RDA0043367	-	-	1	2
<i>Microsphaeropsis</i>			1	10	-	-
<i>Microsphaeropsis</i> sp.	47427	RDA0043366	1	10	-	-
<i>Mucor</i>			1	2	2	4
<i>M. circinelloides</i>	47428	RDA0043365	1	2	-	-
<i>M. circinelloides/racemosus</i>	47430	RDA0043434	-	-	1	2
<i>M. racemosus</i>	47431	RDA0043433	-	-	1	2

Table 2. Continued

Fungal species	KACC No.	Sequence No. <sup>a</sup>	Outside air		Inside air	
			No. of factories <sup>b</sup>	Total concentrations <sup>c</sup> (CFU/m <sup>3</sup> )	No. of factories <sup>b</sup>	Total concentrations <sup>c</sup> (CFU/m <sup>3</sup> )
<i>Penicillium</i>			4	291	5	1,820
<i>P. atramentosum</i>	47432	RDA0043432	-	-	1	10
<i>P. chrysogenum</i> complex	47434	RDA0043430	-	-	2	976
<i>P. citrinum</i>	47435	RDA0043429	-	-	1	20
<i>P. crustosum</i>	47436	RDA0043428	-	-	1	180
<i>P. glabrum</i>	47438	RDA0043426	2	100	3	110
<i>P. glandicola</i>	47439	RDA0043425	1	10	-	-
<i>P. herquei</i>	47440	RDA0043424	1	1	-	-
<i>P. malacaense</i>	47441	RDA0043423	-	-	1	22
<i>P. ochrochloron</i>	47442	RDA0043422	1	90	-	-
<i>P. oxalicum</i>	47443	RDA0043421	-	-	1	10
<i>P. paraherquei</i>	47445	RDA0043419	-	-	1	10
<i>P. paxilli</i>	47446	RDA0043418	1	2	-	-
<i>P. polonicum</i>	47447	RDA0043417	-	-	1	8
<i>P. ramulosum</i>	47448	RDA0043416	-	-	1	2
<i>P. sclerotiorum</i>	47449	RDA0043415	-	-	1	10
<i>P. simplicissimum</i>	47450	RDA0043414	-	-	1	10
<i>P. solitum</i>	47451	RDA0043413	-	-	1	10
<i>P. steckii</i>	47452	RDA0043412	1	30	1	60
<i>P. thomii</i>	47453	RDA0043411	-	-	1	40
<i>P. westlingii</i>	47454	RDA0043410	1	30	-	-
<i>Penicillium</i> sp.			2	28	4	342
<i>Peniophora</i>			-	-	1	40
<i>P. aurantiaca</i>	47455	RDA0043409	-	-	1	40
<i>Periconia</i>			-	-	1	10
<i>Periconia</i> sp.	47457	RDA0043407	-	-	1	10
<i>Pestalotiopsis</i>			1	1	2	22
<i>Pestalotiopsis</i> sp.	47458	RDA0043406	1	1	2	22
<i>Peziza</i>			-	-	1	10
<i>Peziza</i> sp.	47459	RDA0043405	-	-	1	10
<i>Phlebiopsis</i>			-	-	1	20
<i>P. gigantea</i>	47460	RDA0043404	-	-	1	20
<i>Phoma</i>			4	140	3	64
<i>Phoma</i> sp.	47461	RDA0043403	4	140	3	64
<i>Phomopsis</i>			1	8	-	-
<i>Phomopsis</i> sp.	47462	RDA0043402	1	8	-	-
<i>Pichia</i>			-	-	1	110
<i>P. burtonii</i>	47463	RDA0043401	-	-	1	110
<i>Rhizopus</i>			2	3	2	22
<i>R. microsporus</i>	47464	RDA0043400	1	2	-	-
<i>R. stolonifer</i>	47465	RDA0043399	1	1	1	20
<i>Rhizopus</i> sp.	47466	RDA0043398	-	-	1	2
<i>Schizophyllum</i>			2	100	1	20
<i>S. commune</i>	47467	RDA0043397	2	100	1	20
<i>Scopulariopsis</i>			3	25	1	30
<i>S. brevicaulis</i>	47468	RDA0043396	3	25	1	30
<i>Sistotrema</i>			1	120	-	-
<i>S. brinkmannii</i>	47469	RDA0043395	1	120	-	-
<i>Syncephalastrum</i>			-	-	1	10
<i>S. monosporum</i>	47470	RDA0043394	-	-	1	10
<i>Talaromyces</i>			-	-	1	10
<i>Talaromyces</i> sp.	47471	RDA0043393	-	-	1	10

<sup>a</sup>The Rural Development Administration (RDA) numbers are DNA sequence accession numbers from the Korean Agricultural Culture Collection (KACC). Readers can access the sequences at the KACC homepage (<http://www.genebank.go.kr>) using the corresponding KACC numbers.

<sup>b</sup>The numbers indicates factories from which the species were isolated from 6 (outside air) and 5 (inside air) factories.

<sup>c</sup>The numbers indicates the sum of the maximum concentrations of each factory among concentrations on each media.

**Table 3.** List of fungi on *Meju* and their isolation frequencies from air of *Meju* fermentation room, rice straw, and soybeans

Scientific name	Incidence on <i>Meju</i> <sup>a</sup>	Air of <i>Meju</i> fermentation room				Rice straw <sup>b</sup>		Soybeans <sup>c</sup>	
		Outside air		Inside air		No. of factories	Maximum isolation frequency (%)	No. of factories	Maximum isolation frequency (%)
		No. of factories <sup>d</sup>	Total concentration (CFU/m <sup>3</sup> ) <sup>e</sup>	No. of factories <sup>d</sup>	Total concentration (CFU/m <sup>3</sup> ) <sup>e</sup>				
<i>Aspergillus oryzae</i>	***	2	3	4	1,396	10	31.9	7	7.2
<i>Mucor circinelloides</i>	***	1	2	-	-	1	2.1	-	-
<i>Mucor racemosus</i>	***	-	-	1	2	-	-	-	-
<i>Penicillium polonicum</i>	***	-	-	1	8	5	11.8	4	4.2
<i>Eurotium repens</i>	***	2	18	3	200	9	27.1	6	26.8
<i>Scopulariopsis brevicaulis</i>	***	3	25	1	30	-	-	-	-
<i>Penicillium solitum</i>	***	-	-	1	10	2	0.7	-	-
<i>Eurotium chevalieri</i>	***	1	30	1	10	12	25	3	3.2
<i>Lichtheimia ramosa</i>	***	-	-	-	-	2	1.4	2	3.8
<i>Fusarium asiaticum</i>	**	4	43	2	80	11	56.3	1	0.2
<i>Penicillium paneum</i>	**	-	-	-	-	-	-	-	-
<i>Eurotium amstelodami</i>	**	-	-	-	-	7	15.3	1	0.2
<i>Aspergillus niger</i>	**	1	3	4	292	7	9.7	-	-
<i>Penicillium roqueforti</i>	**	-	-	-	-	-	-	1	0.2
<i>Paecilomyces variotii</i>	**	-	-	-	-	-	-	-	-
<i>Cladosporium tenuissimum</i>	**	1	490	1	310	1	6.9	2	23.8
<i>Rhizopus stolonifer</i>	**	1	1	1	20	1	0.7	-	-
<i>Eurotium herbariorum</i>	**	2	54	2	280	5	22.9	8	44
<i>Penicillium crustosum</i>	**	-	-	1	180	-	-	-	-
<i>Penicillium bialowiezense</i>	**	-	-	-	-	-	-	-	-
<i>Eurotium rubrum</i>	**	-	-	-	-	5	16	3	3.8
<i>Lichtheimia corymbifera</i>	**	-	-	-	-	3	13.9	1	0.2
<i>Aspergillus acidus</i>	**	-	-	-	-	-	-	-	-
<i>Aspergillus tamarii</i>	**	-	-	-	-	1	5.6	-	-
<i>Aspergillus fumigatus</i>	**	2	112	1	2	3	4.2	1	0.2
<i>Aspergillus flavus</i>	**	-	-	-	-	2	3.5	-	-
<i>Rhizopus delemar</i>	**	-	-	-	-	-	-	-	-
<i>Scopulariopsis candida</i>	**	-	-	-	-	-	-	-	-
<i>Cladosporium cladosporioides</i>	**	1	1,360	2	380	10	48.6	1	9
<i>Lichtheimia ornata</i>	**	-	-	-	-	-	-	-	-
<i>Aspergillus tubingensis</i>	**	2	2	1	10	10	37.5	2	0.8
<i>Rhizomucor pusillus</i>	**	-	-	-	-	1	1.4	-	-
<i>Mucor mucedo</i>	**	-	-	-	-	-	-	-	-
<i>Aspergillus nidulans</i>	**	-	-	1	450	7	8.3	1	0.4
<i>Penicillium commune</i>	**	-	-	-	-	-	-	-	-
<i>Penicillium palitans</i>	**	-	-	-	-	-	-	-	-
<i>Aspergillus sydowii</i>	**	1	2	3	220	4	4.9	4	3.4
<i>Monascus ruber</i>	**	-	-	-	-	-	-	-	-
<i>Geotrichum silvicola</i>	**	-	-	-	-	-	-	-	-
<i>Aspergillus versicolor</i>	**	-	-	2	160	2	6.3	4	5.6
<i>Neurospora intermedia</i>	**	-	-	-	-	-	-	-	-
<i>Aspergillus ochraceus</i>	**	-	-	1	20	3	4.2	4	2.6
<i>Fusarium sp.</i>	**	2	31	-	-	9	5.6	5	12.8
<i>Penicillium chrysogenum</i>	**	-	-	2	976	1	0.7	2	1.2
<i>Mucor lusitanicus</i>	**	-	-	-	-	-	-	-	-
<i>Rhizopus oryzae</i>	**	-	-	-	-	2	4.9	-	-
<i>Epicoccum nigrum</i>	**	3	26	2	12	3	14.6	1	0.2
<i>Eurotium echinulatum</i>	**	-	-	-	-	1	2.8	3	0.8
<i>Fusarium cf. incarnatum</i>	*	-	-	-	-	4	11.1	2	2.2
<i>Fusarium graminearum</i>	*	-	-	-	-	-	-	-	-
<i>Mucor hiemalis</i>	*	-	-	-	-	-	-	-	-
<i>Penicillium brevicompactum</i>	*	-	-	-	-	2	1.4	-	-

Table 3. Continued

Scientific name	Incidence on <i>Meju</i> <sup>a</sup>	Air of <i>Meju</i> fermentation room				Rice straw <sup>b</sup>		Soybeans <sup>c</sup>	
		Outside air		Inside air		No. of factories	Maximum isolation frequency (%)	No. of factories	Maximum isolation frequency (%)
		No. of factories <sup>d</sup>	Total concentration (CFU/m <sup>3</sup> ) <sup>e</sup>	No. of factories <sup>d</sup>	Total concentration (CFU/m <sup>3</sup> ) <sup>e</sup>				
<i>Aspergillus cibarius</i>	*	-	-	-	-	-	1	0.8	
<i>Cladosporium velox</i>	*	-	-	-	2	3.5	-	-	
<i>Syncephalastrum racemosum</i>	*	-	-	1	10	3.5	1	0.2	
<i>Aspergillus candidus</i>	*	-	-	-	-	-	-	-	
<i>Eurotium tonophilum</i>	*	-	-	-	-	-	1	4	
<i>Penicillium coprophilum</i>	*	-	-	-	-	-	-	-	
<i>Penicillium steckii</i>	*	1	30	1	60	14.6	5	6.6	
<i>Trichosporon</i> sp.	*	-	-	-	-	-	-	-	
<i>Aspergillus parasiticus</i>	*	-	-	-	-	-	-	-	
<i>Cladosporium sphaerospermum</i>	*	-	-	-	1	0.7	1	0.2	
<i>Paecilomyces formosus</i>	*	-	-	-	-	-	-	-	
<i>Penicillium expansum</i>	*	-	-	-	-	-	2	0.6	
<i>Penicillium oxalicum</i>	*	-	-	1	10	1.4	1	4	
<i>Chaetomium cruentum</i>	*	-	-	-	-	-	-	-	
<i>Fusarium acuminatum</i>	*	-	-	1	20	4.2	-	-	
<i>Penicillium carneum</i>	*	-	-	-	-	-	-	-	
<i>Penicillium cyclopium</i>	*	-	-	-	-	-	1	0.4	
<i>Phycomyces blakesleanus</i>	*	-	-	-	-	-	-	-	
<i>Cladosporium varians</i>	*	-	-	-	-	-	-	-	
<i>Allantophomopsis</i> cf. <i>lycopodina</i>	*	-	-	-	-	-	-	-	
<i>Alternaria</i> sp.	*	3	112	2	72	5.6	7	12	
<i>Arthrinium phaeospermum</i>	*	-	-	-	3	2.1	1	3.8	
<i>Aspergillus tritici</i>	*	-	-	-	-	-	-	-	
<i>Aspergillus westerdijkiae</i>	*	1	11	-	3	6.3	3	6	
<i>Botryotinia fuckeliana</i>	*	-	-	-	-	-	-	-	
<i>Botrytis</i> sp.	*	-	-	-	-	-	1	0.2	
<i>Chaetomium</i> sp.	*	-	-	-	3	4.9	2	0.6	
<i>Cladosporium funiculosum</i>	*	-	-	-	-	-	-	-	
<i>Cladosporium fusiforme</i>	*	-	-	-	1	0.7	-	-	
<i>Cladosporium perangustum</i>	*	-	-	-	-	-	-	-	
<i>Fusarium fujikuroi</i>	*	-	-	-	5	9	5	19.6	
<i>Fusarium thapsinum</i>	*	-	-	-	-	-	-	-	
<i>Geotrichum</i> sp.	*	-	-	-	-	-	-	-	
<i>Lichtheimia hyalospora</i>	*	-	-	-	-	-	-	-	
<i>Mucor fragilis</i>	*	-	-	-	-	-	-	-	
<i>Mucor irregularis</i>	*	-	-	-	-	-	-	-	
<i>Neurospora sitophila</i>	*	-	-	-	-	-	-	-	
<i>Penicillium chermesinum</i>	*	-	-	-	-	-	-	-	
<i>Penicillium glabrum</i>	*	2	100	3	110	0.7	-	-	
<i>Penicillium griseofulvum</i>	*	-	-	-	-	-	-	-	
<i>Penicillium hispanicum</i>	*	-	-	-	2	4.2	-	-	
<i>Penicillium nordicum</i>	*	-	-	-	-	-	-	-	
<i>Penicillium olsonii</i>	*	-	-	-	-	-	-	-	
<i>Phoma</i> sp.	*	4	140	3	64	13.9	5	1.8	
<i>Streptobotrys</i> cf. <i>streptothrix</i>	*	-	-	-	-	-	-	-	
<i>Ulocladium</i> sp.	*	-	-	-	-	-	-	-	

<sup>a</sup>The species were isolated from *Meju*, with \*\*\*high frequency, \*\*medium frequency, or \*low frequency.

<sup>b</sup>The number of factories indicates factories from which the species were isolated from 12 factories. Maximum isolation frequency indicates maximum isolation frequency (among 144 pieces of rice straw) among 9 different isolation conditions [8].

<sup>c</sup>The number of factories indicates factories from which the species were isolated from 10 factories. Maximum isolation frequency indicates maximum isolation frequency (among 500 kernels) of untreated soybeans among 3 different media [9].

<sup>d</sup>The number indicates factories from which the species were isolated from 6 (outside air) and 5 (inside air) factories.

<sup>e</sup>The numbers indicate the sum of the maximum concentrations of each factory among concentrations on each media.

*Meju* fermentation rooms were 1,524 CFU/m<sup>3</sup> and 736 CFU/m<sup>3</sup> (Table 1), respectively, and they were identified into 37 genera and 90 species (Table 2). The fungal concentration in inside air of *Meju* fermentation room is similar or lower than that of green area in Seoul, Korea (average, 1,892 CFU/m<sup>3</sup> in winter) [12] and is similar to that of low clean zone of other food product manufacturing plants (average, 2,600 CFU/m<sup>3</sup>) [13]. In this study, there was no significant difference of mycobiota according to *Jang* factories.

In the 6 outside air samples, 27 genera and 50 species were found (Table 2). The concentration of the genus *Cladosporium* was significantly high, followed by those of *Eurotium*, *Penicillium*, *Aspergillus*, *Sistotrema*, and *Schizophyllum* were followed (Table 2). *Cl. cladosporioides* and *Cladosporium* sp. were the dominant species, followed by *Cl. tenuissimum*, *Eurotium* sp., *Sistotrema brinkmannii*, *Asp. fumigatus*, *Schizophyllum commune*, *Phoma* sp., and *Alternaria* sp. However, *Cl. tenuissimum*, *Sis. brinkmannii*, *Asp. fumigatus*, and *Sch. commune* were detected only in 1 or 2 factories. In addition, almost all the other fungi except *Arthrinium sacchari*, *Epicoccum nigrum*, and *S. brevicaulis*, which were detected in 3 *Jang* factories, were also detected only in 1 or 2 factories.

Of the 32 genera and 72 species from the 5 inside air samples, the genus *Aspergillus*, *Cladosporium*, *Penicillium*, and *Eurotium* were collected with high concentrations (Table 2). *A. oryzae* and *Cladosporium* sp. were the dominant species and were detected in 5 and 4 factories, respectively. In addition, *Pen. chrysogenum* (found in 2 factories), *C. cladosporioides* (2), *Asp. niger* (4), *Eur. herbariorum* (2), *Aspergillus* sp. (3), *Penicillium* sp. (4), and *Asp. sydowii* (3) were frequently detected in inside air of the fermentation rooms. Although *Eur. repens*, *Pen. glabrum*, *Phoma* sp., *Bjerkandera adusta*, and *Beauveria bassiana* were infrequently detected in inside air of the fermentation rooms, they were detected in 3 factories. The other species detected in inside air samples were detected in only 1 or 2 factories.

The mycobiota of outside and inside air of the fermentation rooms differed (Table 2), and fungi detected from inside air were more diverse. Seventy-two species were detected from the inside air, whereas 50 species were detected from the outside air. The species belong to the genus *Alternaria*, *Arthrinium*, *Chaetomium*, *Cladosporium*, *Phoma*, and *Schizophyllum* were present in higher concentrations in outside air than in inside air. In particular, *Alternaria*, *Arthrinium*, and *Cladosporium* were significantly higher. Therefore, these fungi present in the inside air may have come in from outside when the inside air was ventilated. However, many fungi belonging to the genus *Aspergillus*, *Penicillium*, and *Eurotium* were frequently detected in inside air but were rarely or not detected in outside air. These observations indicate that these fungi were not influenced by the outside mycobiota but may have been influenced by previous *Meju* fermentation, because they

frequently occurred on *Meju*.

The species, *Asp. oryzae*, *M. circinelloides*, *M. racemosus*, *Pen. polonicum*, *Eur. repens*, *Scopulariopsis brevicaulis*, *Pen. solitum*, *Eur. chevalieri*, and *L. ramosa* were the main species from *Meju* (Table 3). *A. oryzae*, *P. polonicum*, *E. repens*, *P. solitum*, *E. chevalieri*, and *F. asiaticum* are detected on rice straw with high frequency but are rarely isolated from outside air of *Jang* factories. Therefore, most of these could be transferred from rice straw to *Meju*, and they grow and produce abundant spores during *Meju* fermentation, and then as their spores would accumulated inside air of fermentation room, they could influence mycobiota of *Meju* fermentation in the following year. This could explain why concentrations of *Aspergillus*, *Eurotium*, and *Penicillium* in inside air of *Meju* fermentation room is much higher than those of outside air.

*Lichtheimia ramosa*, *M. circinelloides*, *M. racemosus*, and *S. brevicaulis* were rarely or not detected in rice straw and soybean. However, they were detected from outside air of *Meju* fermentation room, although their frequencies were not high, and they are generally known as common fungi in indoor environments [14]. Therefore, *L. ramosa*, *M. circinelloides*, *M. racemosus*, and *S. brevicaulis* on *Meju* might originate from outside (or inside) air of *Meju* fermentation room.

In this study, all main species on *Meju* except *L. ramosa* were detected both inside and outside of fermentation room. However, *L. ramosa* is usually known as an indoor fungus [14]. After all, *Meju* could be provided with almost fungi from air in and out *Meju* fermentation room, which is used for *Meju* fermentation for more than one time.

Rice straw comes into direct contact with soybeans in *Meju*, and so the fungi on rice straw can move to *Meju* and grow on it. Therefore, this has a great influence on *Meju* mycobiota. Rice straw could provide *Meju* with useful fungi for fermentation but also could provide unwanted fungi such as *Fusarium asiaticum*. Traditional *Meju* fermentation is composed of drying at a low temperature (low temperature fermentation process) and fermenting at a high temperature and humidity (high temperature fermentation process) [4]. *F. asiaticum* usually originates from rice straw and grows on it during fermentation at low temperatures. Therefore, in order to avoid contamination of *F. asiaticum*, a method could be developed to use rice straw only during high temperature fermentation. Without rice straw, *Meju* might be provided with main fungi from both inside and outside of *Meju* fermentation room during the low temperature fermentation process, if the fermentation room is used for *Meju* fermentation more than once.

## ACKNOWLEDGEMENTS

This work was partly supported by a research project (no. PJ00866601) of the National Academy of Agricultural Science (NAAS), Rural Development Administration, Republic of Korea.

## REFERENCES

1. Byun YG, Kim SH, Joo HK, Lee GS, Yim MH. Isolation and identification of protease producing bacteria, *Bacillus subtilis* YG-95 from the traditional *Me-Ju* and its production conditions. *Agric Chem Biotechnol* 1998;41:342-8.
2. Han J, Kim HJ, Lee SS, Lee IS. Inhibitive effects of *Meju* extracts made with a single inoculum of the fungi isolated from the traditional *Meju* on the human leukemia cell line. *Kor J Mycol* 1999;27:312-7.
3. Lee SS, Park KH, Choi KJ, Won SA. A study in hyphomycetous fungi found on maejus, a raw material of Korean traditional soysources. *Kor J Mycol* 1993;21:247-72.
4. Hong SB, Kim DH, Lee M, Baek SY, Kwon SW, Houbraken J, Samson RA. *Zygomycota* associated with traditional *Meju*, a fermented soybean starting material for soy sauce and soybean paste. *J Microbiol* 2012;50:386-93.
5. Hong SB, Kim DH, Lee M, Baek SY, Kwon SW, Samson RA. Taxonomy of *Eurotium* species isolated from *Meju*. *J Microbiol* 2011;49:669-74.
6. Kim DH, Lee M, Baek SY, Lee JK, Samson RA, Hong SB. Identification and extracellular enzyme activities of *Penicillium* strains isolated from *Meju*. *J Microbiol* (in press).
7. Lee SS. *Meju* fermentation for a raw material of Korean traditional soy products. *Kor J Mycol* 1995;23:161-75.
8. Hong SB, Kim DH, Samson RA. *Aspergillus* associated with *Meju*, a fermented soybean starting material for traditional soy sauce and soybean paste in Korea. *Mycobiology* (in press).
9. Choi KS, Lee HJ, Kwon DJ. Physicochemical and microbiological properties of Korean traditional *Meju*. *Korean J Food Preserv* 2009;16:217-22.
10. Kim DH, Kim SH, Kwon SW, Lee JK, Hong SB. Fungal diversity of rice straw for *Meju* fermentation. *J Microbiol Biotechnol* 2013;23:1654-63.
11. Kim DH, Kim SH, Kwon SW, Lee JK, Hong SB. Mycoflora of soybeans used for *Meju* fermentation. *Mycobiology* 2013;41:100-7.
12. Kim KY, Kim D. Distribution characteristics of airborne fungi in a partial area of Seoul city. *J Environ Health Sci* 2012;38:407-14.
13. Gwak HJ, Lee HJ, Lee SH, Na HJ. Identification and concentration of airborne microbes in food manufacturing plants. *J Fd Hyg Safety* 2011;26:361-5.
14. Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B. Food and indoor fungi. Utrecht: CBS-KNAW Fungal Biodiversity Center; 2010. p. 10-8.