

Fungal Diversity of Rice Straw for *Meju* Fermentation

Dae-Ho Kim^{1,2}, Seon-Hwa Kim¹, Soon-Wo Kwon¹, Jong-Kyu Lee², and Seung-Beom Hong^{1*}

¹Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-853, Republic of Korea

²Tree Pathology and Mycology Laboratory, Division of Forest Environment Protection, Kangwon National University, Chuncheon 200-701, Republic of Korea

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*Corresponding author
Phone: +82-31-299-1866;
Fax: +82-31-299-1869;
E-mail: funguy@korea.kr

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Rice straw is closely associated with *meju* fermentation and it is generally known that the rice straw provides *meju* with many kinds of microorganisms. In order to elucidate the origin of *meju* fungi, the fungal diversity of rice straw was examined. Rice straw was collected from 12 *Jang* factories where *meju* are produced, and were incubated under nine different conditions by altering the media (MEA, DRBC, and DG18), and temperature (15°C, 25°C, and 35°C). In total, 937 strains were isolated and identified as belonging to 39 genera and 103 species. Among these, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, and *Penicillium* were the dominant genera. *Fusarium asiaticum* (56.3%), *Cladosporium cladosporioides* (48.6%), *Aspergillus tubingensis* (37.5%), *A. oryzae* (31.9%), *Eurotium repens* (27.1%), and *E. chevalieri* (25.0%) were frequently isolated from the rice straw obtained from many factories. Twelve genera and 40 species of fungi that were isolated in the rice straw in this study were also isolated from *meju*. Specifically, *A. oryzae*, *C. cladosporioides*, *E. chevalieri*, *E. repens*, *F. asiaticum*, and *Penicillium polonicum* (11.8%), which are abundant species in *meju*, were also isolated frequently from rice straw. *C. cladosporioides*, *F. asiaticum*, and *P. polonicum*, which are abundant in the low temperature fermentation process of *meju* fermentation, were frequently isolated from rice straw incubated at 15°C and 25°C, whereas *A. oryzae*, *E. repens*, and *E. chevalieri*, which are abundant in the high temperature fermentation process of *meju* fermentation, were frequently isolated from rice straw incubated at 25°C and 35°C. This suggests that the mycobiota of rice straw has a large influence in the mycobiota of *meju*. The influence of fungi on the rice straw as feed and silage for livestock, and as plant pathogens for rice, are discussed as well.

Keywords: Fungi, *Meju*, mycobiota, rice straw

Introduction

Rice straw is the most abundant agricultural residue in the world [24]. It is utilized as feed and bedding for animals, a source of biofuel, an additive of compost, and media for mushroom cultivation [1, 8, 15, 24, 26]. Diverse microorganisms such as bacteria, fungi, and yeast are present in the rice straw and these are utilized in various ways [8]. Silage is fermented by the colonizing microorganisms on rice straw, which aids in the process of digestion in the livestock [26]. The microorganisms on rice straw are used during the fermentation of *meju* and *cheonggukjang* [8, 17].

Many fungi possess efficient enzymes and they are useful for food and feed industries [25].

Meju fermentation is closely associated with rice straw, as the rice straw is in direct contact with the soybeans in *meju* during the fermentation process, and it is known that the rice straw provides *meju* with various kinds of microorganisms [17]. The fungus on rice straw was examined by Lee *et al.* [15] in Korea and by Heral [7] in Egypt, but the isolation and identification of the fungi from rice straw in both studies were not comprehensive. The aims of this study were to examine the fungal diversity on the rice straw for *meju* fermentation, to compare the mycobiota

inhabiting the rice straw and *meju*, and to presume the origin of fungi in *meju*. In addition, we were keen to document the basic information about the mycobiota for industrial usage of rice straw.

Materials and Methods

Rice straw samples for *meju* fermentation were collected from 12 *Jang* factories where *meju* are produced from November to December 2011. The geographical origin of the rice straw is listed in Table 1. After dividing the rice straw into three parts, the top, middle, and bottom, they were cut into pieces approximately 2 cm in length. Four pieces (2 pieces per plate) of each rice straw sample was incubated on nine different conditions using three media such as malt extract agar (MEA), dichloran rose bengal chloramphenicol agar (DRBC), and dichloran 18% glycerol agar (DG18) medium, and incubating at three different temperatures (15°C, 25°C, and 35°C) in the dark for 5–7 days. In total, 1,296 pieces of rice straw (12 samples; 3 parts, 3 temperatures, 3 media, and each 4 pieces) were used. The fungal colonies that grew on the rice straw or media were transferred to new MEA or DG18 media (for xerophilic fungi) and were further incubated for several days. After confirmation by microscopic examination, the fungi were transferred to MEA or DG18 slants, and were maintained at 4°C.

For the molecular identification of the various fungi, genomic DNA was extracted using the DNeasy Plant Mini Kit (69106; Qiagen, Hilden, Germany) according to the method of Kim et al. [13]. The selection of the sequencing region depended on the genera; the partial β -tubulin gene using bt2a and bt2b primers [6] for *Aspergillus*, *Eurotium*, and *Penicillium*, the partial actin gene using act-512F and act-783R primers [2] for *Cladosporium*, the partial elongation factor 1-alpha gene using EF1 and EF2 primers [5] for *Fusarium*, and the internal transcribed spacer of nuclear ribosomal DNA (rDNA-ITS) using ITS1 and ITS4 primers [28] for the other genera. In addition, morphological characters of strains were analyzed for confirmation of molecular identification, according to methods by Pitt and Hocking [19] and Samson et al. [20].

Results and Discussion

A total of 937 strains were isolated from 1,296 pieces of rice straw and were identified as belonging to 39 genera and 103 species (Table 2). The genera *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, and *Penicillium* were the dominant fungi (occurrence more than 100 in 1,296 pieces) in rice straw, followed by the genera *Phoma*, *Epicoccum*, *Lichtheimia*, *Nigrospora*, *Chaetomium*, *Bionectria*, and *Trichothecium* (occurrence more than 30/1,296 pieces). *Cochliobolus* and *Coprinellus* were infrequently isolated (occurrence less than 30/1,296 pieces) but were isolated from 7 *Jang* factories.

Table 1. List of the rice straw used in this study.

No.	Location	Incidence of fungi (%)
1	Chungbuk Seocheon	99 ^a
2	Chungnam Gongju	100
3	Gangwon Yangyang	76
4	Gyeongbuk Chilgok	90
5	Gyeonggi Anseong	98
6	Gyeonggi Icheon	82
7	Gyeonggi Yangpyeong	86
8	Gyeonggi Yongin	98
9	Jeonbuk Buan	100
10	Jeonbuk Sunchang D	88
11	Jeonbuk Sunchang H	94
12	Jeonnam Damyang	81

^aThe percentage indicates rice straw pieces from which the species were isolated, out of 108 pieces (3 temperatures, 3 media, 3 parts, and 4 pieces) of rice straw.

Occurrences of fungi from rice straw were dependent on the isolation media used (Table 2). The most diverse species were isolated on DRBC, followed by DG18. *Aspergillus* (except *Eurotium*) and *Cladosporium* were isolated from rice straw cultured on all the three media, MEA, DRBC, and DG18. *Eurotium*, *Schizophyllum*, *Syncephalastrum*, and *Penicillium* were more frequently isolated on DG18, but the other genera were more frequently isolated on MEA and/or DRBC. Specifically, *Beauveria*, *Bionectria*, *Coprinellus*, *Hypochnicium*, *Khuskia*, *Rhizomucor*, *Talaromyces*, and *Verticillium* were not isolated on DG18. Occurrence of fungi from rice straw was also dependent on incubation temperatures (Table 2). The most diverse species were isolated from samples incubated at 25°C, but some fungi were isolated only from samples incubated at 15°C or 35°C. However, the mycobiota on rice straw did not differ much according to collecting regions and parts of rice straw (Table 2). Therefore, the isolation frequency of the fungal species from the rice straw was counted based on the optimum media and temperature in this study (Table 2, fourth column). The total pieces of rice straw were 144 (12 samples, 3 parts, and each 4 pieces) on optimum isolating condition for every species.

Fourteen species of *Aspergillus* were isolated (Table 2). *A. tubingensis* and *A. oryzae* were isolated from rice straw obtained from 10 factories with a 37.5% frequency (54/144 pieces) and 31.9% (46/144) under optimum isolating conditions, respectively. Many *Aspergillus* species showed higher frequency in samples incubated at 35°C than at 25°C

Table 2. List of fungal species isolated from rice straw and their frequencies.

Scientific name	No. of factories ^a	Incidence on <i>meju</i> ^b	Isolation frequency on optimum isolating condition		Media ^e			Temperatures ^f			Parts of rice straw ^g			Representative strain and its DNA sequence	
			Incidence (%) ^c	Optimum condition ^d	MEA	DRBC	DG18	15°C	25°C	35°C	Top	Middle	Bottom	KACC no.	Sequence no. ^h
Alternaria	6				10	6	4	9	7	4	13	7	-		
<i>A. alternata</i> sp.	6		5.6	15M	10	6	4	9	7	4	13	7	-	47264	RDA0041956
Arthrinium	4				4	1	4	6	2	1	3	3	3		
<i>A. phaeospermum</i>	3	*	2.1	15G	1	1	3	3	1	1	1	2	2	47265	RDA0041959
<i>A. sacchari</i>	2		2.8	15M	3	-	1	3	1	-	2	1	1	47266	RDA0041960
Aspergillus	12				139	145	155	50	109	280	138	147	154		
<i>A. aculeatus</i>	1		1.4	25R	-	4	3	-	3	4	1	4	2	47267	RDA0041961
<i>A. creber</i>	1		2.1	15R	-	3	2	5	-	-	4	-	1	47268	RDA0041962
<i>A. flavus</i>	2	**	3.5	35M/G	5	3	5	-	-	13	2	3	8	47269	RDA0041963
<i>A. fumigatus</i>	3	**	4.2	15M	11	4	1	7	-	9	3	7	6	47270	RDA0041964
<i>A. nidulans</i>	7	**	8.3	35G	10	17	12	-	11	28	16	11	12	47271	RDA0041965
<i>A. niger</i>	7	**	9.7	35G	10	11	17	-	7	31	15	11	12	47272	RDA0041966
<i>A. ochraceus</i>	3	**	4.2	25R	4	6	3	-	12	1	5	4	4	47273	RDA0041967
<i>A. oryzae</i>	10	***	31.9	35M	69	43	63	9	40	126	55	56	64	47274	RDA0041968
<i>A. sydowii</i>	4	**	4.9	25R/35G	9	16	13	10	15	13	17	17	4	47275	RDA0041969
<i>A. tamaritii</i>	1	**	5.6	15R	7	8	-	15	-	-	5	4	6	47276	RDA0041970
<i>A. terreus</i>	4		2.8	35R	1	4	1	-	-	6	3	3	-	47277	RDA0041971
<i>A. tubingensis</i>	10	**	37.5	35G	30	40	70	1	23	116	31	48	61	47278	RDA0041972
<i>A. versicolor</i>	2	**	6.3	25G	3	5	9	-	17	-	3	9	5	47279	RDA0041973
<i>A. westerdijkiae</i>	3	*	6.3	25R/G	5	9	13	3	23	1	9	8	10	47280	RDA0041974
<i>Aspergillus</i> sp.	1		0.7	35R	-	1	-	-	-	1	-	1	-	47281	RDA0041975
Beauveria	1				1	2	-	-	3	-	1	2	-		
<i>B. bassiana</i>	1		1.4	25R	1	2	-	-	3	-	1	2	-	47282	RDA0041976
Bionectria	3				15	19	-	6	26	2	7	16	11		
<i>B. epichloe</i>	1		2.1	25R	3	4	-	-	5	2	2	4	1	47283	RDA0041977
<i>Bionectria</i> sp.	2		8.3	25R	12	15	-	6	21	-	5	12	10	47284	RDA0041978
Bjerkandera	1				-	2	-	-	-	2	1	-	1		
<i>B. adusta</i>	1		1.4	35R	-	2	-	-	-	2	1	-	1	47285	RDA0041979
Chaetomium	6				12	20	5	7	23	7	11	16	10		
<i>C. globosum</i>	5		5.6	25M	10	6	-	1	12	3	6	9	1	47286	RDA0041980
<i>Chaetomium</i> sp.	3		4.9	25R	3	14	3	6	12	2	4	8	8	47287	RDA0041981

Table 2. Continued.

Scientific name	No. of factories ^a	Incidence on <i>meji</i> ^b	Isolation frequency on optimum isolating condition			Media ^e			Temperatures ^f			Parts of rice straw ^g			Representative strain and its DNA sequence	
			Incidence (%) ^c	Optimum condition ^d	MEA	DRBC	DG18	15°C	25°C	35°C	Top	Middle	Bottom	KACC no.	Sequence no. ^h	
<i>Circinella</i>	1				-	1	-	-	1	-	-	1	-	-		
<i>C. muscae</i>	1		0.7	25R	-	1	-	-	1	-	-	1	-	-	47288	RDA0041982
<i>Cladosporium</i>	12	**			115	160	139	194	216	4	153	132	129			
<i>C. cladosporioides</i>	10	**	48.6	15R	91	136	117	176	167	1	122	110	112		47289	RDA0041983
<i>C. fusiforme</i>	1		0.7	15G	-	-	1	1	-	-	1	-	-		47290	RDA0041984
<i>C. pseudocladosporioides</i>	2		13.2	25R	14	19	12	-	45	-	17	15	13		47291	RDA0041985
<i>C. sphaerospermum</i>	1	*	0.7	25G	-	-	1	-	1	-	1	-	-		47292	RDA0041986
<i>C. tenuissimum</i>	1	**	6.9	15M	10	7	-	17	-	-	7	6	4		47293	RDA0041987
<i>C. velox</i>	2	*	3.5	25G	-	-	8	-	5	3	6	2	-		47294	RDA0041988
<i>Clonostachys</i>	1				1	1	-	-	2	-	-	2	-			
<i>Clonostachys</i> sp.	1		0.7	25M/R	1	1	-	-	2	-	-	2	-		47295	RDA0042060
<i>Cochliobolus</i>	7				5	14	3	2	6	14	11	6	5			
<i>C. geniculatus</i>	1		1.4	35M	2	-	-	-	-	2	2	-	-		47296	RDA0041989
<i>C. lunatus</i>	1		0.7	15R	-	1	-	1	-	-	-	1	-		47297	RDA0041990
<i>C. miyabeanus</i>	2		2.8	25R	2	4	-	1	4	1	5	1	-		47298	RDA0041991
<i>C. spicifer</i>	1		2.1	35R	-	3	-	-	-	3	-	1	2		47299	RDA0041992
<i>Cochliobolus</i> sp.	5		4.2	35R	2	6	3	-	2	9	5	3	3		47300	RDA0041993
<i>Coprinellus</i>	7				2	18	-	1	6	13	2	8	10			
<i>C. radians</i>	6		7.6	35R	-	16	-	-	5	11	2	6	8		47301	RDA0041994
<i>C. xanthothrix</i>	1		0.7	15M	1	-	-	1	-	-	-	1	-		47302	RDA0041995
<i>Coprinellus</i> sp.	1		1.4	35R	1	2	-	-	1	2	-	1	2		47303	RDA0041996
<i>Cytospora</i>	1				-	-	1	1	-	-	-	1	-			
<i>C. mali</i>	1		0.7	15G	-	-	1	1	-	-	-	1	-		47304	RDA0041997
<i>Daldinia</i>	1				-	1	-	1	-	-	1	-	-			
<i>D. childiae</i>	1		0.7	15R	-	1	-	1	-	-	1	-	-		47305	RDA0041998
<i>Epicoccum</i>	3	**			30	30	2	42	19	1	26	20	16			
<i>E. nigrum</i>	3	**	14.6	15R	30	29	2	42	19	-	25	20	16		47306	RDA0041999
<i>Epicoccum</i> sp.	1		0.7	35R	-	1	-	-	-	1	1	-	-		47307	RDA0042000
<i>Eurotium</i>	12				23	29	188	59	88	93	75	82	83			
<i>E. amstelodami</i>	7	**	15.3	35G	-	1	30	5	4	22	8	13	10		47308	RDA0042001

Table 2. Continued.

Scientific name	No. of factories ^a	Incidence on <i>mejiu</i> ^b	Isolation frequency on optimum isolating condition		Media ^e			Temperatures ^f			Parts of rice straw ^g			Representative strain and its DNA sequence	
			Incidence (%) ^c	Optimum condition ^d	MEA	DRBC	DG18	15°C	25°C	35°C	Top	Middle	Bottom	KACC no.	Sequence no. ^h
<i>E. checalteri</i>	12	***	25.0	25G	23	26	73	32	44	46	42	38	42	47309	RDA0042002
<i>E. echinulatum</i>	1	**	2.8	25G	-	-	4	-	4	-	-	-	4	47310	RDA0042003
<i>E. herbariorum</i>	5	**	22.9	25G	-	-	37	4	33	-	14	10	13	47311	RDA0042004
<i>E. heterocaryoticum</i>	3		4.9	25G	-	2	12	-	7	7	3	9	2	47312	RDA0042005
<i>E. cf. medium</i>	3		2.1	25G	-	-	4	1	3	-	1	1	2	47313	RDA0042006
<i>E. repens</i>	9	***	27.1	25G	-	1	91	23	40	29	31	34	27	47314	RDA0042007
<i>E. rubrum</i>	5	**	16.0	35G	-	-	43	4	16	23	12	14	17	47315	RDA0042008
<i>Eurotium</i> sp.	1		1.4	15G	-	-	2	2	-	-	-	1	1	47316	RDA0042009
Fusarium	12				173	193	121	218	229	40	144	166	177		
<i>F. acuminatum</i>	1	*	4.2	25R	-	6	4	-	10	-	2	3	5	47317	RDA0042010
<i>F. andiyazi</i>	1		1.4	35G	-	-	2	-	-	2	-	2	-	47318	RDA0042011
<i>F. asiaticum</i>	11	***	56.3	15R	146	159	98	193	197	13	121	136	146	47319	RDA0042012
<i>F. cf. incarnatum</i>	4	*	11.1	25M	19	24	21	19	42	3	23	23	18	47320	RDA0042013
<i>F. concentricum</i>	2		0.7	15/25G	-	-	2	1	1	-	-	2	-	47321	RDA0042014
<i>F. oxysporum</i>	2		2.8	35R	3	4	-	-	-	7	3	4	-	47322	RDA0042015
<i>F. fujikuroi</i>	5	*	9.0	15M	23	25	7	25	10	20	15	21	19	47323	RDA0042016
<i>Fusarium</i> sp.	9		5.6	15R	9	14	3	11	10	5	7	10	9	47324	RDA0042017
Ganoderma	1				-	1	-	-	-	1	-	-	1		
<i>Ganoderma</i> sp.	1		0.7	35R	-	1	-	-	-	1	-	-	1	47325	RDA0042018
Hypochnicium	1				4	2	-	2	2	2	-	3	3		
<i>Hypochnicium</i> sp.	1		1.4	25M	4	2	-	2	2	2	-	3	3	47326	RDA0042019
Hypoxylon	1				-	1	-	-	-	1	-	1	-		
<i>Hypoxylon</i> sp.	1		0.7	35R	-	1	-	-	-	1	-	1	-	47327	RDA0042020
Irpex	2				12	9	1	-	6	16	10	7	5		
<i>Irpex</i>	2		6.3	35M	12	9	1	-	6	16	10	7	5	47328	RDA0042021
Kluskia	3				5	-	-	2	3	-	-	2	3		
<i>Kluskia</i>	3		2.1	25M	5	-	-	2	3	-	-	2	3	47329	RDA0042022
Lichtheimia	5				11	22	16	2	3	44	14	14	21		
<i>Lichtheimia</i>	5		13.9	35R	8	20	14	2	1	39	11	12	19	47330	RDA0042023
L. ramosa	2				3	2	2	-	2	5	3	2	2	47331	RDA0042024

Table 2. Continued.

Scientific name	No. of factories ^a	Incidence on <i>meji</i> ^b	Isolation frequency on optimum isolating condition		Media ^e			Temperatures ^f			Parts of rice straw ^g			Representative strain and its DNA sequence	
			Incidence (%) ^c	Optimum condition ^d	MEA	DRBC	DG18	15°C	25°C	35°C	Top	Middle	Bottom	KACC no.	Sequence no. ^h
<i>Microascus</i>	3				9	13	4	-	-	26	7	6	13		
<i>Microascus</i> sp.	3		9.0	35R	9	13	4	-	-	26	7	6	13	47332	RDA0042025
<i>Mucor</i>	1				1	3	-	-	-	4	2	-	2		
<i>M. circinelloides</i>	1	***	2.1	35R	1	3	-	-	-	4	2	-	2	47333	RDA0042026
<i>Myrothecium</i>	1				-	1	-	-	-	1	-	-	1		
<i>Myrothecium</i> sp.	1		0.7	35R	-	1	-	-	-	1	-	-	1	47334	RDA0042027
<i>Nigrospora</i>	5				13	18	8	8	31	-	8	21	10		
<i>N. oryzae</i>	5		9.0	25R	13	18	8	8	31	-	8	21	10	47335	RDA0042028
<i>Penicillium</i>	10				61	63	91	63	96	56	72	77	66		
<i>P. brevicompactum</i>	2	*	1.4	15G/25M/R	2	2	2	2	4	-	2	3	1	47336	RDA0042035
<i>P. chrysogenum</i> complex	1	**	0.7	35G	-	-	1	-	-	1	1	-	-	47337	RDA0042034
<i>P. citrinum</i>	2		4.9	35G	-	4	7	-	-	11	5	5	1	47338	RDA0042033
<i>P. copticola</i>	1		0.7	15G	-	-	1	1	-	-	1	-	-	47339	RDA0042032
<i>P. glabrum</i>	2	*	0.7	15./25R/G	1	1	1	1	2	-	1	1	1	47341	RDA0042030
<i>P. hispanicum</i>	2	*	4.2	35G	-	1	8	-	2	7	2	2	5	47342	RDA0042029
<i>P. oxalicum</i>	2	*	1.4	25G	1	-	4	1	2	2	1	4	-	47343	RDA0042036
<i>P. picum</i>	1	**	6.3	35R/G	1	9	9	-	-	19	7	8	4	47344	RDA0042037
<i>P. polonicum</i>	5	***	11.8	15M	17	12	28	35	19	3	22	19	16	47345	RDA0042038
<i>P. solitum</i>	2	***	0.7	15M/G	1	-	1	2	-	-	1	1	-	47346	RDA0042039
<i>P. steckii</i>	4	*	14.6	25R	16	24	27	5	56	6	25	23	19	47347	RDA0042040
<i>P. sumatrense</i>	1		5.6	25G	-	-	13	-	8	5	7	2	4	47348	RDA0042041
<i>P. thomii</i>	2		1.4	25M	2	-	1	1	2	-	1	-	2	47349	RDA0042042
<i>P. toxocarum</i>	1		4.9	25M	7	-	-	-	7	-	4	3	-	47350	RDA0042043
<i>P. verrucosum</i>	1		3.5	35R	1	5	-	-	-	6	3	2	1	47340	RDA0042031
<i>Penicillium</i> sp.	6		10.4	15M	23	18	3	22	17	5	9	15	20	47351	RDA0042044
<i>Peroneutypa</i>	1				2	-	-	-	2	-	-	2	-		
<i>P. scoparia</i>	1		1.4	25M	2	-	-	-	2	-	-	2	-	47352	RDA0042045
<i>Pestalotiopsis</i>	1				2	-	2	2	2	-	1	-	3		
<i>Pestalotiopsis</i> sp.	1		0.7	15M/G, 25M/G	2	-	2	2	2	-	1	-	3	47353	RDA0042046

Table 2. Continued.

Scientific name	No. of factories ^a	Incidence on <i>meji</i> ^b	Isolation frequency on optimum isolating condition		Media ^e			Temperatures ^f			Parts of rice straw ^g			Representative strain and its DNA sequence		
			Incidence (%) ^c	Optimum condition ^d	MEA	DRBC	DG18	15°C	25°C	35°C	Top	Middle	Bottom	KACC no.	Sequence no. ^h	
<i>Phlebiopsis</i>	1				1	1	-	-	2	-	2	-	-	-		
<i>P. gigantea</i>	1		0.7	25M/R	1	1	-	-	2	-	2	-	-	-	47354	RDA0042047
<i>Phoma</i>	7				26	34	3	37	26	-	27	22	14			
<i>P. herbarum</i>	1		1.4	15M	2	-	-	2	-	-	-	2	-	-	47355	RDA0042048
<i>Phoma</i> sp.	6		13.9	15R	24	34	3	35	26	-	27	20	14	47356	RDA0042049	
<i>Rhizomucor</i>	2				5	-	-	3	2	2	2	-	3			
<i>R. pusillus</i>	1	**	1.4	35M	2	-	-	-	2	2	2	-	-	47357	RDA0042050	
<i>R. variabilis</i>	1		2.1	25M	3	-	-	3	-	-	-	-	3	47358	RDA0042051	
<i>Rhizopus</i>	3				20	6	11	5	8	24	10	17	10			
<i>R. arrhizus</i>	2		4.2	25M	8	2	6	1	7	8	5	10	1	47359	RDA0042052	
<i>R. oryzae</i>	2	**	4.9	35M	11	4	5	3	1	16	5	6	9	47360	RDA0042053	
<i>R. stolonifer</i>	1	**	0.7	15M	1	-	-	1	-	-	-	1	-	47361	RDA0042054	
<i>Schizophyllum</i>	2				2	5	6	-	-	-	13	4	5	4		
<i>S. commune</i>	2		4.2	35G	2	5	6	-	-	-	13	4	5	4	47362	RDA0042055
<i>Syncephalastrum</i>	2				2	1	5	-	-	8	4	2	2			
<i>Syncephalastrum</i> sp.	2		3.5	35G	2	1	5	-	-	8	4	2	2	47363	RDA0042056	
<i>Talaromyces</i>	2				1	3	-	-	-	4	2	1	1			
<i>Talaromyces</i> sp.	2		2.1	35R	1	3	-	-	-	4	2	1	1	47364	RDA0042057	
<i>Trichothecium</i>	4				17	14	3	11	22	1	19	14	1			
<i>T. roseum</i>	4		7.6	25M	17	14	3	11	22	1	19	14	1	47365	RDA0042058	
<i>Verticillium</i>	1				2	3	-	3	1	1	3	1	1			
<i>Verticillium</i> sp.	1		1.4	15R	2	3	-	3	1	1	3	1	1	47366	RDA0042059	

^aThe number indicates factories from which the species were isolated.

^bThe species were isolated from *meji*, **with high frequency, **medium frequency, or ³low frequency.

^cThe number indicates the incidence of each species on optimum isolating condition from 144 pieces (12 samples, 3 parts, and each 4 pieces) of rice straw.

^dNumbers 15, 25, and 35 indicate the temperature at which the samples were incubated, and the letters M, R, and G indicate the media MEA, DRBC, and DG18 used for the culture, respectively.

^eThe number indicates rice straw pieces from which the species were isolated, out of 432 pieces (12 samples, 3 temperatures, 3 parts, and 4 pieces) of rice straw.

^fThe number indicates rice straw pieces from which the species were isolated, out of 432 pieces (12 samples, 3 media, 3 parts, and 4 pieces) of rice straw.

^gThe number indicates rice straw pieces from which the species were isolated, out of 432 pieces (12 samples, 3 temperatures, and 4 pieces) of rice straw.

^hThe RDA numbers are the DNA sequence accession number of Korean Agricultural Culture Collection (KACC). Readers can access the sequence from information of the corresponding KACC No. in the KACC homepage (<http://www.genebank.go.kr>).

or 15°C. Among the six *Cladosporium* species (Table 2) isolated, the most frequent species was *C. cladosporioides* (48.6%), followed by *C. pseudocladosporioides*. *Cladosporium* strains were frequently isolated from rice straw samples cultured at either 15°C or 25°C. In the case of *Eurotium*, eight species (Table 2) were isolated from rice straw. *E. repens* (27.1%) was the most frequent species, followed by *E. chevalieri* (25%), *E. herbariorum* (22.9%), *E. rubrum* (16%), and *E. amstelodami* (15.3%). *Eurotium* was isolated with a higher frequency at 25°C or 35°C, than at 15°C. Fifteen species of *Penicillium* were isolated from rice straw (Table 2) and the common species were *P. polonicum* (11.8%), *P. steckii* (14.6%), and *Penicillium* sp. (10.4%). *P. polonicum* was present in the highest frequency in samples of rice straw incubated at 15°C, whereas *P. steckii* was the predominant species in samples incubated at 25°C. *Penicillium* is generally known as psychrophilic fungi [21]. Eight species of *Fusarium* were isolated from rice straw and the predominant species was *F. asiaticum* (11 factories with 56.3% frequency), which showed higher frequency at 15°C and 25°C. Among the other genera, *Epicoccum nigrum* (14.6%), *Lichtheimia corymbifera* (13.9%), and *Phoma* sp. (13.9%) were also isolated with relatively high frequency. In this study, the genera *Aspergillus*, *Cladosporium*, and *Eurotium* were isolated from the rice straw obtained from all the 12 factories. *Aspergillus oryzae*, *A. tubingensis*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. repens*, and *F. asiaticum* were consistently isolated from many factories and with high frequency, and these species could be the typical species on rice straw.

Lee et al. [15] isolated 16 genera and 25 species from rice straw collected from 11 different sites in Korea. *Curvularia intermedia* was the most frequent fungi, followed by *Alternaria alternata*, *Dothideomycete* sp., *Cladosporium* sp., *Nigrospora oryzae*, *Aspergillus niger*, *Fusarium graminearum*, *Mucor circinelloides*, *Gaeumannomyces graminis* var. *graminis*, *Penicillium oxalicum*, *Trichoderma harzianum*, and *Fusarium proliferatum*. Of these, 6 genera and 10 species such as *Aspergillus niger*, *A. ochraceus*, *A. versicolor*, *Cochliobolus miyabeanus*, *Epicoccum nigrum*, *Fusarium graminearum* (*F. asiaticum* in this study), *Fusarium oxysporum*, *Mucor circinelloides*, *Nigrospora oryzae*, and *Penicillium oxalicum* were also isolated in this study. Among the dominant species reported by Lee et al. [15], *Alternaria alternata*, *Curvularia intermedia*, *F. proliferatum*, *Gaeumannomyces graminis* var. *graminis*, and *Trichoderma harzianum* were not isolated in this study. On the other hand, the dominant fungi isolated from the rice straw in this study, *Aspergillus oryzae*, *A. tubingensis*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, and *E. repens* were not isolated in the study of Lee et al. [15].

In the study carried out by Helal [7], 30 genera and 64 species were isolated from rice straw collected from the Sharkia Province in Egypt. *Aspergillus terreus*, *A. niger*, *A. fumigatus*, *A. flavus*, *Mucor racemosus*, *Myrothecium roridum*, and *Trichoderma koningii* were the frequently isolated species. Among the fungi reported by Helal [7], 12 genera and 21 species, including *Aspergillus flavus* (*A. oryzae* or *A. flavus* in this study), *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, and *Epicoccum nigrum*, were also isolated from this study. However, *Mucor racemosus*, *Myrothecium roridum*, and *Trichoderma koningii*, which were the dominant species in the study of Helal [7], were not isolated in this study. On the other hand, *Aspergillus tubingensis*, *Cladosporium pseudocladosporioides*, *Eurotium* spp., *Fusarium asiaticum*, *F. incarnatum*, *Lichtheimia corymbifera*, *Penicillium polonicum*, and *P. steckii* were not reported from the rice straw samples in the study of Helal [7].

The difference in mycoflora between this and the other studies [7, 15] could probably be due to the different collection period of the rice straw and the isolation media used. Lee et al. [15] collected rice straw shortly after harvest, whereas this study collected in the winter season, 2-4 months after harvest. Therefore, the rice straw used in this study was drier and more decomposed than that of Lee et al. [15]. Xerophilic and dry resistant fungi were isolated more in this study. Helal [7] and Lee et al. [15] did not isolate *Eurotium*, as they used only MEA or Czapek's agar, respectively, but did not use DG18 or DRBC. *Eurotium* does not grow on MEA or Czapek's agar, but grows well on DG18 [21]. DG18 and DRBC, which were used in this study, are the recommended media for isolation and enumeration of fungi from foods and feeds [21]. Lee et al. [15] used morphological characters and rDNA-ITS for identification, whereas Helal [7] used only morphological characters for identification. Nowadays, many fungi are not able to be clearly identified by morphological character and/or rDNA-ITS sequence [22]. Specifically, *A. niger/tubingensis* and *A. sydowii/versicolor* cannot be distinguished by rDNA-ITS, but the β -tubulin sequence can distinguish them [18, 23].

From the traditional Korean *meju*, which is made by using rice straw, 26 genera and 101 species of fungi were isolated [9–12, 14]. Of these, 12 genera and 40 species were also isolated from rice straw. *Aspergillus oryzae*, *Mucor circinelloides*, *M. racemosus*, *Penicillium polonicum*, *Eurotium repens*, *Scopulariopsis brevicaulis*, *P. solitum*, *E. chevalieri*, *Lichtheimia ramosa*, *Fusarium asiaticum*, and *Cladosporium cladosporioides* were the most frequently isolated from traditional Korean *meju*. Of the above, *Aspergillus oryzae* (31.9% of rice straw), *Eurotium chevalieri* (25.0%), *E. repens*

(27.1%), *Fusarium asiaticum* (56.3%) *Penicillium polonicum* (11.8%), and *Cladosporium cladosporioides* (48.6%) were also frequently isolated from rice straw. *C. cladosporioides*, *F. asiaticum*, and *P. polonicum*, which are abundant in the low temperature fermentation process of *meju* fermentation, were frequently isolated from rice straw incubated at 15°C and 25°C in this study. *A. oryzae*, *E. repens*, and *E. chevalieri*, which are abundant in the high temperature fermentation process of *meju* fermentation, were frequently isolated from rice straw incubated at 25°C and 35°C. This suggests that the rice straw could provide *meju* with diverse fungi during its fermentation process. Indeed, it has been observed that many fungi are transferred from rice straw to *meju*. The fungi, *C. cladosporioides* and *F. asiaticum*, which could be transferred from rice straw to *meju* during the low temperature fermentation process, are not regarded as ferment fungi, but are regarded as contaminants of *meju*. On the other hand, *A. oryzae*, *E. repens*, and *E. chevalieri*, which could be transferred into *meju* from rice straw during the high temperature fermentation process, are generally regarded as good ferment fungi on *meju*. In other words, rice straw might provide *meju* with contaminant fungi during the low temperature fermentation process and with beneficial fungi during the high temperature fermentation process. Therefore, it might be a good method to use rice straw during only high temperature fermentation, in order not to provide *meju* with contaminant fungi, but to provide *meju* with beneficial fungi, if we consider only the fungal perspective.

The most frequent fungi on rice straw, *Fusarium asiaticum* (56.3%), is a causal pathogen of *Fusarium* blight on rice. *Cladosporium cladosporioides* (48.6%), the second most frequent species on rice straw, has been reported as the causal pathogen of ear blight on rice [27]. In addition, *Fusarium incarnatum* (ear blight) (11.1%), *Cochliobolus lunatus* (ear blight) (0.7%), *Khuskia oryzae* (ear blight) (2.1%), *F. fujikuroi* (Bakanae disease) (9.0%), *Nigrospora oryzae* (Brown spot) (9.0%), and *Cochliobolus miyabeanus* (Helminthosporium blight) (2.8%) [27] were also isolated from rice straw. This information could be useful to understand the mechanism of disease occurrence on rice.

Sung *et al.* [26] detected mycotoxins from 42% of rice straw bale silage. Ochratoxin A, deoxynivalenol, and zearalenone were detected, but aflatoxin (B₁, B₂, G₁, G₂) and fumonisin (B₁, B₂) were not detected [26]. *Aspergillus ochraceus* and *A. westerdijkiae*, which were isolated quite frequently in this study, could be causal fungi for the ochratoxin, and *Fusarium asiaticum*, which was also one of the most frequent species, also could be a causal fungus for deoxynivalenol

and zearalenone [26]. However, *Aspergillus flavus*, which was isolated with 3.5% frequency in this study, did not produce a detectable quantity of aflatoxin in the study of Sung *et al.* [26].

On the other hand, enzymes such as amylase, xylanase, and protease that are produced by fungi that are found on the rice straw are useful in silage preparation [16]. In addition, the fungi isolated from rice straw, *Aspergillus oryzae* (Koji), *Eurotium herbariorum* (Katsuobushi), *E. repens* (Katsuobushi), *Penicillium chrysogenum* (animal food), *Rhizopus arrhizus* (Kaffir Beer), and *Rhizopus oryzae* (Lao-chao, biodiesel fuel), are used widely in various industries [3, 4]. Therefore, the documentation of fungal diversity and their frequency in rice straw in this study can provide the basic information for usage of rice straw for various industries.

Strains isolated in this study are preserved in the Korean Agricultural Culture Collection (KACC) (<http://www.genebank.go.kr>) for future research and these are accessible to other researchers.

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