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Zygomycota Associated with Traditional Meju, a Fermented Soybean Starting Material for Soy Sauce and Soybean Paste

Seung-Beom Hong[1]*, Dae-Ho Kim[1], Mina Lee[1], Seong-Yeol Baek[2], Soon-wo Kwon[1], Jos Houbraken[3], and Robert A. Samson[3]

[1]Korean Agricultural Culture Collection, and [2]Fermentation and Food Processing Division, National Academy of Agricultural Science, RDA, Suwon 441-707, Republic of Korea
[3]CBS-KNAW Fungal Biodiversity Centre, 3508AD Utrecht, The Netherlands

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Various zygomycota species were detected during a study of the mycobiota of meju, a brick of dried fermented soybeans, used in Korean cuisine. Two hundred and sixty-eight strains were isolated from 98 finished meju products collected in various regions of Korea from 2009 to 2011, and 96 strains were isolated from in-process meju on various farms from 2010 to 2011. The isolated zygomycota were identified using phenotypic characteristics combined with DNA sequences of the internal transcribed spacer regions of ribosomal DNA and the D1/D2 nuclear ribosomal large subunit. Of 364 zygomycota strains, 108 were identified as *Mucor circinelloides*, 96 as *M. racemosus*, 60 as *Lichtheimia ramosa*, 22 as *Rhizopus stolonifer*, 16 as *Lichtheimia corymbifera*, and the other 62 strains comprised 10 other species. The psychrotrophic species, *Mucor circinelloides* and *M. racemosus* were predominantly present during low temperature fermentation (LTF) and the thermotolerant species *Lichtheimia ramosa* and *Rhizomucor* species were predominant during high temperature fermentation (HTF). The results suggest that temperature has a large influence on the zygomycota composition during the fermentation process of meju.

Keywords: meju, zygomycota, *Mucor circinelloides, Mucor racemosus, Lichtheimia ramosa*

Introduction

Korean traditional meju (hereinafter meju) is a brick of dried fermented soybeans, which serves as the basis of several Korean condiments, such as doenjang (soybean paste), ganjang (soy sauce) and gochujang (hot pepper paste), which are essential sauces of authentic Korean cuisine. Meju is naturally fermented and various microorganisms, such as bacteria, yeasts and fungi, are involved in the process. Fungi play an important role in the fermentation and degrade macromolecules of the soybeans into small nutrient molecules (Lee, 1995; Lee *et al.*, 1995). However, the fungi of meju have not been determined exactly, and neither have they been preserved in any microbial culture collection (Hong *et al.*, 2011). Therefore, people who work on meju farms, name the fungi by their colors, such as white fungus, yellow fungus, green fungus, black fungus, etc.

Recently, the mycobiota of meju was elucidated and *Eurotium* and *Penicillium* were isolated, identified, and preserved (Hong *et al.*, 2011; Kim *et al.*, 2012). However, the zygomycota were not treated in those studies, although zygomycota species are known to be important organisms in the production in various traditional Asian foods. *Rhizopus oligosporus* is the main producer of tempeh, a traditional soy product in Indonesia. *R. chinensis, R. oryzae*, and *Mucor indicus* are also found in tempeh (Dijksterhuis and Samson, 2006). *Actinomucor repens, A. taiwanensis, Mucor circinelloides, M. hiemalis, M. racemosus*, and *Rhizopus microsporus* were isolated from Sufu Pehtze, Chinese molded tofu (Han *et al.*, 2004). Three species of *Rhizopus* (Yihn and Lee, 1968) and 12 species of zygomycota were reported from meju (Lee *et al.*, 1993, 1995). In the study of Lee *et al.* (1995), *Mucor hiemalis, M. circinelloides* f. *griseo-cyanus, Absidia corymbifera, Rhizopus oryzae, R. stolonifer, Absidia glauca*, and *A. spinosa* were the main microorganisms in meju fermentation, together with *Bacillus* spp. and *Scopulariopsis brevicaulis,* and the zygomycota played an important role in the early stage of meju fermentation when a high moisture level was maintained.

The objectives of this study were: 1) to elucidate which zygomycota occur and which are the main species in meju, 2) to elucidate during which meju process they grow, and 3) to secure zygomycota strains in biological resource collections to provide a starting point for future meju research.

Materials and Methods

To examine which fungi occur in meju products, we collected 98 meju loaves from various regions in Korea as follows: 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 to isolate zygomycota from the meju. To determine during which meju process the fungi grow, we visited four meju farms in Gyeonggi province every week from molding process (the end of November, 2010) to the submergence of meju in brine (the middle of February, 2011). Additionally, we visited

*For correspondence. E-mail: funguy@korea.kr; Tel.: +82-31-299-1866; Fax: +82-31-299-1869

diverse meju farms in Chungnam, Chungbuk, Jeonnam, Jeonbuk, and Gyeongbuk provinces in the middle of February 2011 to examine the mycobiota of meju in the southern part of the Korean Peninsula. We isolated the fungi from in-process meju by direct plating fungi on Malt Extract Agar (MEA) [50 g Malt Extract Agar (Oxoid, UK), 1 L distilled water].

A total of 364 strains of zygomycota were isolated. For molecular identification, the D1/D2 region of the nuclear ribosomal large subunit (hereinafter D1/D2) (primers, NL1 and LR3) (Alastruey-Izquierdo *et al.*, 2010) was sequenced. The D1/D2 sequences of the zygomycota strains from meju were compared with those of Alastruey-Izquierdo *et al.* (2010), Abe *et al.* (2006, 2010), Alvarez *et al.* (2011) for *Lichtheimia*, *Rhizopus*, and *Mucor* species, respectively. Sequences of *Rhizomucor, Syncephalastrum*, and *Phycomyces* species were compared with those of the National Center for Biotechnology Information (NCBI) GenBank. Molecular based identifications were confirmed with phenotypic characteristics. For macro-morphological observations, different types of agar media were used: MEA (at both 25°C and 37°C), DG18 [31.5 g Dichloran-Glycerol Agar Base (Oxoid), 220 g glycerol, 0.1 g chloramphenicol, 1 L distilled water], and OA [72.5 g Oatmeal agar (Difco, USA), 1 L distilled water] (25°C). Microscopic mounts were made from MEA 25°C colonies.

For further characterization, 31 representative strains were selected based on their source and molecular and morphological characteristics (Table 1). To determine the taxonomic positions of these selected strains, the internal transcribed spacer (ITS) of ribosomal DNA was also examined, using primers ITS1 and ITS4 (White *et al.*, 1990). DNA sequences of ITS and D1/D2 from this study were compared with those of Alastruey-Izquierdo *et al.* (2010), Abe *et al.* (2006,

Table 1. Overview of zygomycetous species from Korean traditional meju

Scientific name	This study			Total no. of strains	Representative strains (KACC[c] no.)	ITS GenBank no.	D1/D2 GenBank no.
	From finished meju[a]		From in-process meju[b]				
	Meju no.	Strain no.	Strain no.				
Lichtheimia corymbifera	10	16	0	16	45830	JN315001	JN315032
					46048	JN315002	JN315033
L. hyalospora	1	1	0	1	45835	JN315003	JN315034
L. ornata	5	9	1	10	45837	JN315004	JN315035
					46050	JN315005	JN315036
L. ramosa	27	38	22	60	45849	JN315006	JN315037
					46054	JN315007	JN315038
Mucor circinelloides	45	83	25	108	45855	JN315008	JN315039
					46062	JN315009	JN315040
M. hiemalis	0	0	4	4	46074	JN315010	JN315041
					46072	JN315011	JN315042
M. cf. *irregularis*	0	0	1	1	46073	JN315012	JN315043
M. mucedo	2	3	6	9	46082	JN315013	JN315044
					46084	JN315014	JN315045
M. racemosus	39	75	21	96	45882	JN315015	JN315046
					46090	JN315016	JN315047
M. lusitanicus	2	2	1	3	46078	JN315017	JN315048
M. cf. *lusitanicus*	2	2	0	2	46076	JN315018	JN315049
M. cf. *fragilis*	1	1	0	1	46077	JN315019	JN315050
Phycomyces blakesleeanus	0	0	2	2	46094	JN315020	JN315051
					46095	JN315021	JN315052
Rhizomucor pusillus	3	5	4	9	45886	JN315022	JN315053
					46096	JN315023	JN315054
Rhizopus delemar	6	9	3	12	46099	JN315024	JN315055
					46100	JN315025	JN315056
R. oryzae	2	2	3	5	46101	JN315026	JN315057
					46102	JN315027	JN315058
R. stolonifer	14	18	4	22	46105	JN315031	JN315059
					45890	JN315028	JN315060
Syncephalastrum racemosum	2	2	1	3	46108	JN315029	JN315061
					45894	JN315030	JN315062
total	98	266	98	364			

[a] From finished meju: Zygomycota were isolated from the finished meju product by direct and by dilution plating.
[b] From in-process meju: Zygomycota were isolated from in-process meju on farms by direct plating.
[c] KACC: Korean Agricultural Culture Collection, National Academy of Agricultural Science, Suwon, Korea.

2010), Alvarez *et al.* (2011), and NCBI GenBank. DNA data were analyzed using the Tamura-Nei parameter distance calculation model with gamma-distributed substitution rates, which were then used to construct the neighbor-joining tree with MEGA version 5.1 (Tamura *et al.*, 2011). To determine the support for each clade, bootstrap analysis was performed with 1,000 replications.

For growth characteristics at diverse temperatures, 5 µl spore suspensions of the representative strains were inoculated on MEA plates. The plates were incubated at the following temperatures: 6, 12, 18, 24, 30, 36, 40, 45, and 50°C. Colony diameters were measured after incubating for five days.

Results and Discussion

Identification of meju strains

Recently, species boundaries of several zygomycota genera were investigated using rDNA sequences. Alastruey-Izquierdo *et al.* (2010) established the molecular taxonomy of the genus *Lichtheimia* with D1/D2, ITS, and actin genes. In order to identify *Lichtheimia* meju strains, the D1/D2 DNA sequences of meju strains were compared with those of type or representative strains from Alastruey-Izquierdo *et al.* (2010). Eighty-seven meju strains clustered with type or authentic strains of *Lichtheimia* species on the D1/D2 tree (data not shown). Of these, 60 belonged in the *L. ramosa* cluster, 16

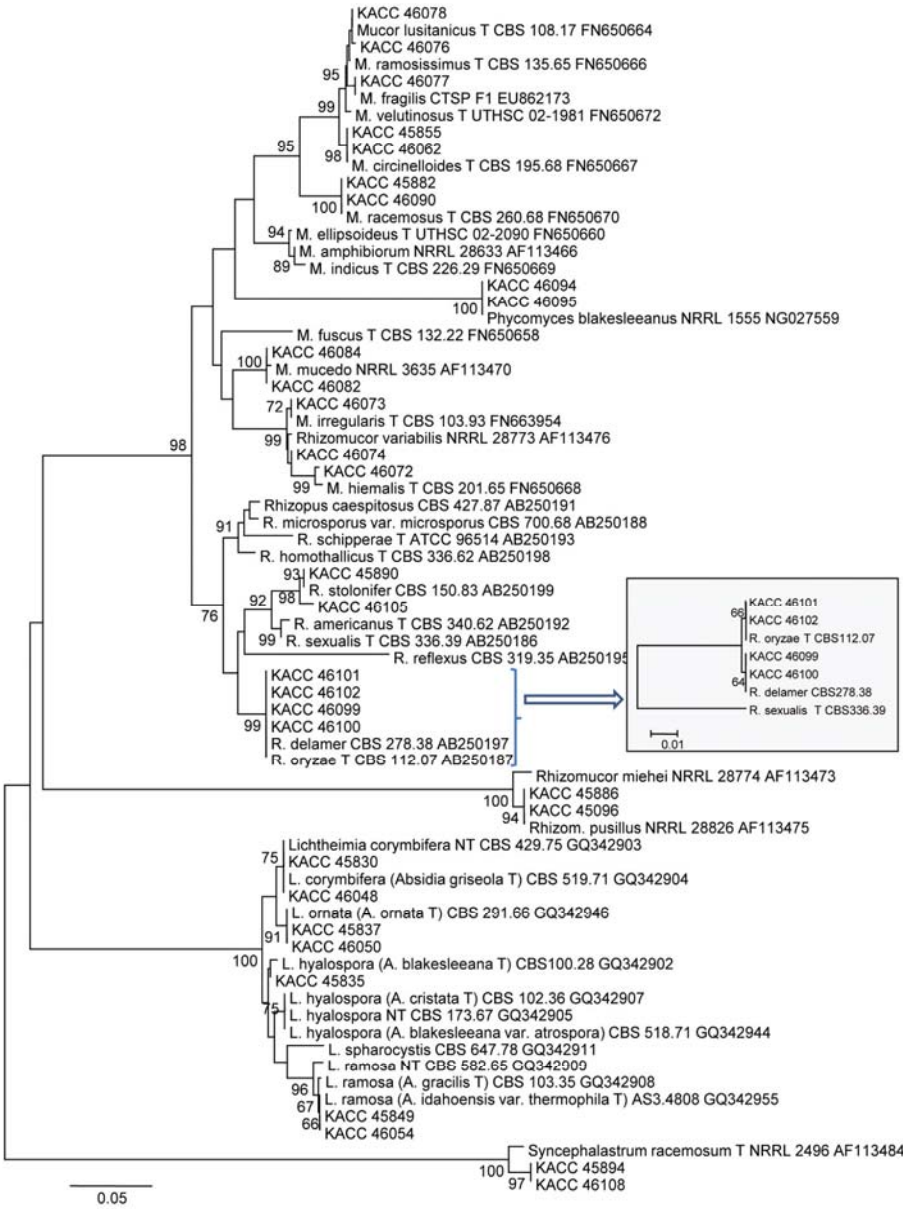

Fig. 1. Neighbor-joining tree depicting taxonomic positions of zygomycetous strains from meju. The tree is based on DNA sequences of the D1/D2 region of the nuclear ribosomal large subunit. Numbers at the nodes are bootstrap values greater than 60 and the subscript (N)T before a strain no. denotes (neo)type strain of the species.

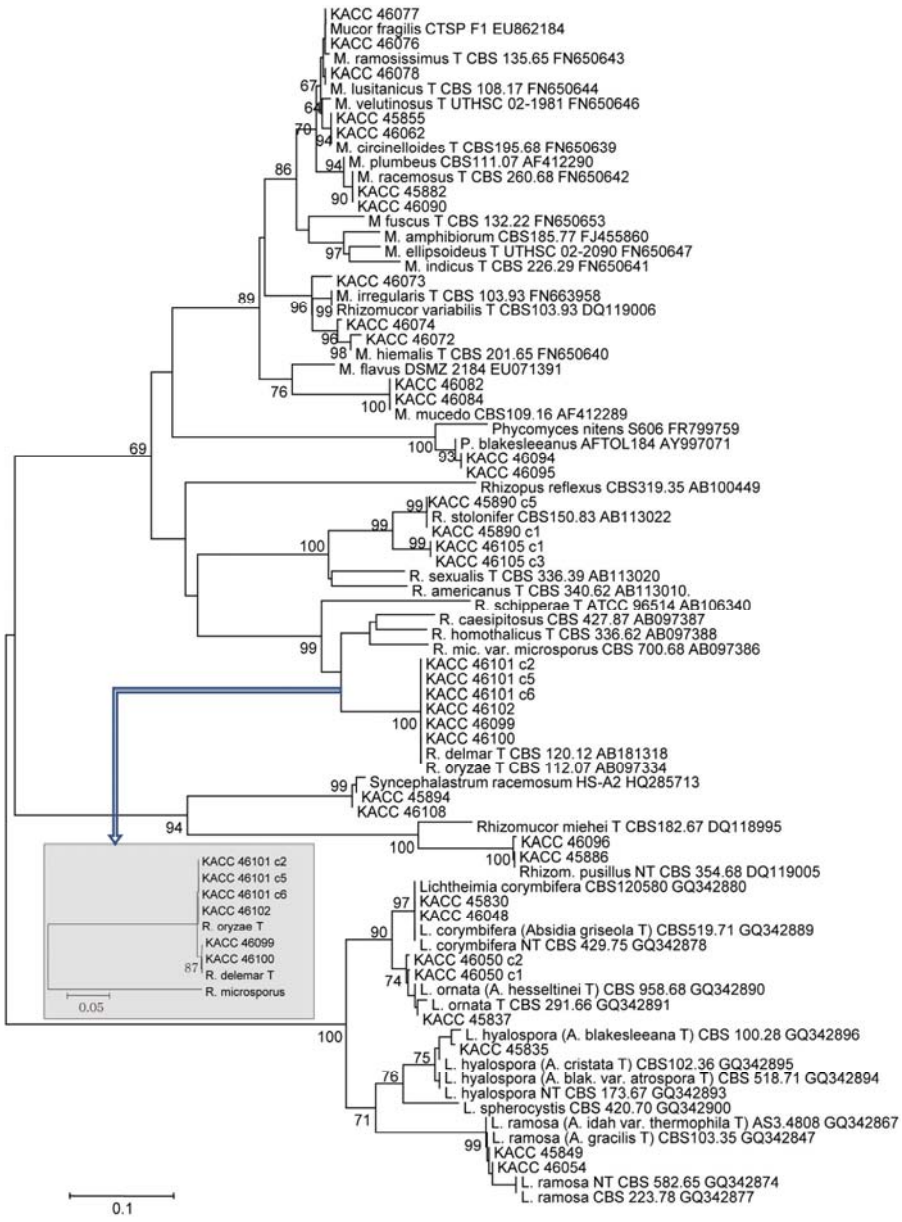

Fig. 2. Neighbor-joining tree depicting taxonomic positions of zygomycetous strains from meju. The tree is based on DNA sequences of the ITS-rDNA. Numbers at the nodes are bootstrap values greater than 60, and the subscript (N)T before a strain no. denotes the (neo)type strain of the species. *C number is the respective number of clones.

in *L. corymbifera*, 9 in *L. ornata* and 1 in *L. hyalospora* (Table 1). The taxonomic position of the representative strains is shown in Figs. 1 and 2 based on D1/D2 and ITS, respectively. Meju strains are unambiguously positioned into reference strains with high bootstrap value. Concordant with Alastruey-Izquierdo *et al.* (2010), morphological characters such as sporangiophore morphology and spore shape were insufficient to differentiate the four *Lichtheimia* meju species. Growth rate at different temperatures also did not differentiate these four species from meju, although *L. ramosa* grew more vigorously at 40°C and 45°C than the other species (Fig. 4). *Lichtheimia* species grew widely on the surface of meju, and sometimes grew on the inside, when the conditions were good (Figs. 3C and D). The fungi grew as white hyphae at first, and became gray with age due to sporangiospore formation.

Alvarez *et al.* (2011) classified *Mucor* as 15 species based on D1/D2 and ITS. In order to identify the 224 *Mucor* meju strains, the D1/D2 DNA sequences were compared with those of type or representative strains from Alvarez *et al.* (2011). Most meju strains clearly clustered with type or authentic strains on the D1/D2 tree (data not shown). One hundred and eight strains clustered into a *M. circinelloides* clade, 96 into *M. racemosus*, and 9 into *M. mucedo*. The taxonomic positions of the representative *Mucor* meju strains are shown in Figs. 1 and 2 based on D1/D2 and ITS, respectively. Meju strains of *M. circinelloides, M. racemosus*, and *M. mucedo* clustered unambiguously with reference strains with high bootstrap values. Phenotypic characters such as size and shape of sporangiospores and columellae,

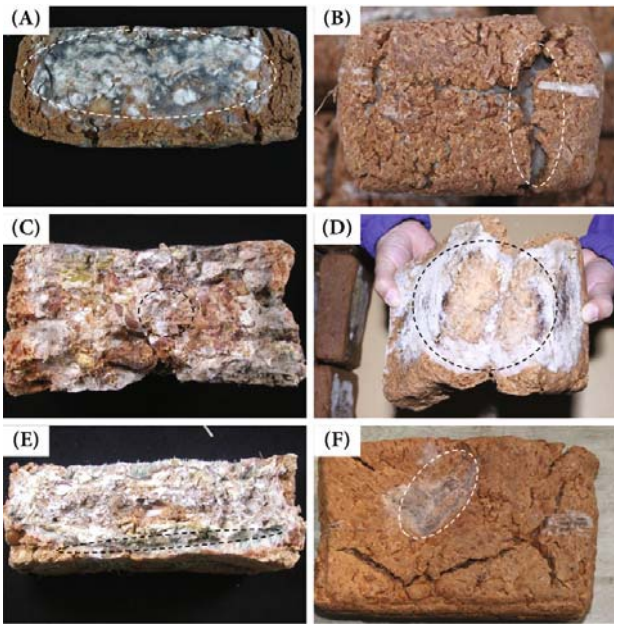

Fig. 3. Zygomycota on meju. (A) *Mucor circinelloides* (M2680), gray to black mold; (B) *Muc. racemosus* (M2605), mold in cracks; (C) *Lichtheimia corymbifera* (KACC 46048), white mold in center region; (D) *Lic. ramosa* (M2550), all white mold in meju; (E) *Rhizopus stolonifer* (M2653), inner side black mold which forms thin layer. Outer green fungus is *Eurotium*; (F) *Rhizomucor pusillus* (M2707), fungus on surface of meju.

existence of chlamydospores, and growth at diverse temperatures, supported their identification. *M. mucedo*, including KACC 46082 and KACC 46084, had much bigger spores (7–17 μm) and columellae (up to 120 μm). *M. circinelloides*, including KACC 45855 and KACC 46062, grew well at 37°C, but *M. mucedo* did not grow at 30°C, and growth of *M. mucedo* on DG18 was very restricted (<16 mm). *M. racemosus*, including KACC 45849 and KACC 46054, had numerous chlamydospores.

Some *Mucor* meju strains, however, showed ambiguous taxonomic positions. KACC 46072 and KACC 46074 clustered with *M. hiemalis* type strain CBS 201.65 on both the D1/D2 and ITS trees (Figs. 1 and 2). However, KACC 46073 showed an ambiguous position between *M. hiemalis* and *M. irregularis* [new name for *Rhizomucor variablis* var. *variablis* by Alvarez *et al.* (2011)] on the ITS tree (Fig. 2) and it clustered with *M. irregularis* type strain CBS 103.93 with a 72% bootstrap value on the D1/D2 tree (Fig. 1). Phenotypically, the five strains showed characteristics of *M. hiemalis* (Schipper, 1973) and were not differentiated from each other. Furthermore, we could not find any rhizoids from KACC 46073 on MEA cultures, although the rhizoid is one of the most important characters of *M. irregularis* (Alvarez *et al.*, 2011). Therefore, we identified KACC 46072 and KACC 46074 as *M. hiemalis,* and named KACC 46073 as *Mucor* cf. *irregularis*.

KACC 46078 clustered with *M. lusitanicus* [new name for *M. circinelloides* f. *lusitanicus* by Alvarez *et al.* (2011)] type strain CBS 108.17 on both the D1/D2 and ITS trees (Figs. 1 and 2). KACC 46076 clustered with *M. lusitanicus* on the LSU tree (Fig. 1), but was positioned ambiguously between *M. lusitanicus* and *M. ramosissimus* on the ITS tree (Fig. 2). KACC 46077 clustered with *M. fragilis* strain CTSP F1 on both ITS and D1/D2 trees. However, the above three strains had variable, ellipsoidal to narrow ellipsoidal (4–11 μm) sporangiospores, which is characteristic of *M. lusitanicus* (Schipper, 1976), and were not differentiated based on phenotypic characteristics. Furthermore KACC 46076 and KACC 46077 were isolated from the same meju loaf. Therefore, we named KACC 46078 as *M. lusitanicus*, KACC 46076 as *M.* cf. *lusitanicus,* and KACC 46077 as *Mucor* cf. *fragilis*. Species differentiation of *M. lusitanicus, M. ramosissimus*, and *M. fragilis* is ambiguous. They have nearly the same ITS and D1/D2 sequences, and they do not have obvious phenotypic characteristics to differentiate them from each other. It was difficult to fit the meju strains of this study into the taxonomic system of Alvarez *et al.* (2011). A better taxonomic system is needed for *Mucor*.

Mucor species were usually found on cracks of meju in which a high level of moisture was maintained. They showed diverse colors on meju, i.e., *M. circinelloides* was white to gray black, *M. racemosus* was white to gray, *M. mucedo* was gray to black, and *M. hiemalis* was white to whitish yellow (Figs. 3A and B).

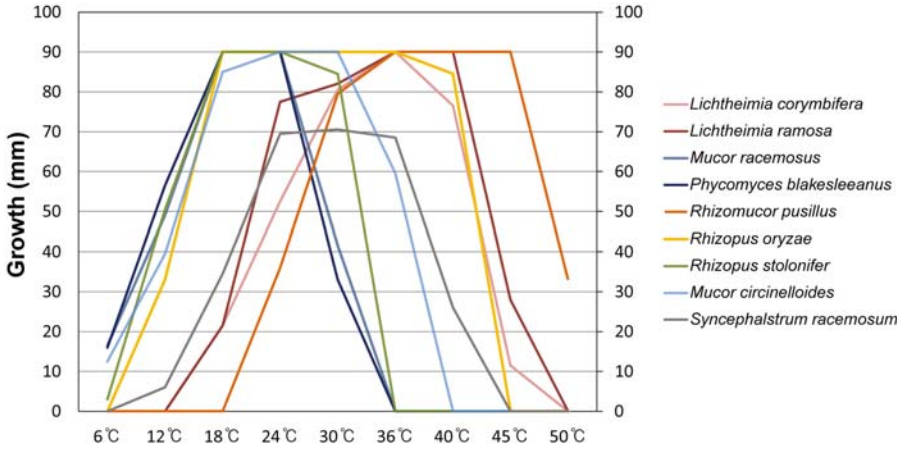

Fig. 4. Growth characteristics of meju borne zygomycota at diverse temperatures. Growth size of representative strains (Table 1) was measured after incubating five days on MEA and the mean value of each species was plotted.

The taxonomy of *Rhizopus* has been well established using molecular methods by Abe *et al.* (2006, 2010) and using morphology by Zheng *et al.* (2007). To identify 39 *Rhizopus* meju strains, D1/D2 DNA sequences were compared with those of type and representative strains from Abe *et al.* (2006, 2010). All *Rhizopus* meju strains clearly clustered with type or authentic strains of Abe *et al.* on the D1/D2 tree (data not shown). Twenty-two strains were gathered into a *R. stolonifer* cluster based on D1/D2 sequence, and the taxonomic positions of representative strains are shown in Figs. 1 and 2 based on D1/D2 and ITS, respectively. Species identification was not a problem for the *R. stolonifer* meju strains. However, it was not easy to sequence the ITS regions of this species, because *R. stolonifer* had different types of ITS fragments in one isolate and we had to clone the PCR product prior to sequencing. *R. stolonifer* was well separated from the other species by morphology. It had rhizoids that grew vigorously and that branched repeatedly, and its sporangiospores were always distinctly striate. Twelve strains of *R. delemar* and five strains of *R. oryzae* were clearly separated by D1/D2 (data not shown), although they showed high sequence similarity (99.7%) and similar morphological characteristics. Taxonomic positions of the representative strains of the two species are shown in Figs. 1 and 2. The two species are not separated from each other because of high similarity (99.7% and 99.3% similarity for D1/D2 and ITS, respectively), but were well separated when the tree was re-made with only close strains (boxes in Figs. 1 and 2). *R. delemar* and *R. oryzae* had simple rhizoids and could grow at 37°C, while *R. stolonifer* had more branching rhizoids and could not grow at 37°C.

On meju, *Rhizopus* species were well separated from the other zygomycota by their black appearance. They were found on a portion of the surface or a layer of the inner part of meju (Fig. 3E).

Nine strains of *Rhizomucor pusillus* were easily identified, because these strains grow well at 50°C (Hoog *et al.*, 2000) and the identification was confirmed by D1/D2 and ITS DNA sequences (Figs. 1 and 2). This fungus was found on the surface, in cracks, and the aerated inner side of meju with gray to black mold (Fig. 3F). *Syncephalastrum racemosum* found in cracks of meju was easily identified by its unique merosporangium (Hoog *et al.*, 2000). Its identification was confirmed by ITS and D1/D2 DNA sequences (Figs. 1 and 2). *Phycomyces blakesleeanus* grew like black hairs both on plates and meju (Benjamin and Hesseltine, 1959) and could also be identified by phenotypic characters. Taxonomic positions of *P. blakesleeanus*, KACC 46094 and KACC 46095 are shown on Figs. 1 and 2.

In this study, six genera comprising 15 species of zygomycota were isolated and identified from various samples of Korean traditional meju. Six of 15 species were also reported by Lee *et al.* (1995) [*Lichtheimia corymbifera* (as *Absidia corymbifera*), *Mucor hiemalis*, *M. circinelloides*, *M. racemosus*, *Rhizopus stolonifer*, and *R. oryzae*] and the other nine species were newly isolated from Korean traditional meju in this study. Although 15 species were isolated from meju, only several species were predominantly present. The most frequently isolated species was *M. circinelloides* (108/364 strains), followed by *M. racemosus* (96), *Lichtheimia ramosa* (60), *Rhizopus stolonifer* (22), *L. corymbifera* (16), *R. delemar* (12), *L. ornata* (10), *Rhizomucor pusillus* (9), *M. mucedo* (9), and the others. According to isolation frequency, *M. circinelloides*, *M. racemosus*, and *L. ramosa* accounted for 73% of all the zygomycota species isolated from meju (264/364 strains) in this study.

Growth characteristics based on temperature

Growth temperature ranges for meju borne zygomycota were clearly different among genera (Fig. 4). *Rhizomucor* could grow at the highest temperature, followed by *Lichtheimia*, *Syncephalastrum*, *Rhizopus*, *Mucor*, and *Phycomyces*. *Rhizomucor* is thermotolerant and grew well from 24°C to 50°C. *Lichtheimia* grew from 12°C to 45°C, and *L. ramosa* could grow better than the other species of the genus at a range of 40–45°C. *Rhizopus* grew from 6°C to 40°C, and the growth temperature range was clearly different between *R. stolonifer* and *R. oryzae*/ *R. delemar*. The latter two species grew well at 40°C, but the former could not grow at 36°C. *Mucor* is a psychrotrophic genus, and all *Mucor* species from meju could grow at 6°C, grew well at 12°C, and showed nearly maximum growth at 18°C. *M. circinelloides* grew well at 36°C and showed vigorous growth at a wide range of temperatures (6–36°C), which could be why it was the most frequently isolated zygomycota species from meju. On the contrary, at 36°C, the other *Mucor* meju species could not grow, or could only grow restrictedly. *M. mucedo* did not grow even at 30°C. Growth at 36°C (or 37°C) is one of the most useful methods to differentiate between *M. circinelloides* and other meju *Mucor* species. *Phycomyces blakesleeanus* is also a psychrotrophic species and it did not grow at 36°C.

Zygomycota flora in the meju fermentation process

Traditional meju fermentation takes 60–90 days. During the first 40–80 days, the meju is dried at low temperatures (low temperature fermentation, LTF) and during the last stage (10–30 days), the meju is maintained under high temperatures and humidity conditions (high temperature fermentation, HTF) (Lee *et al.*, 1995).

The detailed LTF process depends on the farms. Traditionally, shaped meju are hung under the eaves, and dried naturally by the wind during the winter season from November to February. Presently, most farms use a vinyl-house, a glass house, or a room with a Korean floor heating system. Whatever method farms adopt during LTF, the meju is maintained at a low temperature (under 15°C, maximum 20°C) (Table 2). At this temperature, *Mucor*, *Rhizopus*, and *Phycomyces* species can actively grow (Fig. 4). Actually, *Mucor* and *Rhizopus* species were observed and *M. circinelloides* and *M. racemosus* were frequently isolated (Table 2). The fungi grew in clefts of meju and on sides of meju loaves which stood close to each other, where the humidity was maintained at a higher level (Fig. 3B).

In the High Temperature Fermentation (HTF) process, the meju is piled up in several layers and covered with thick cloths, and then the room is heated. During the process, the temperature of the meju environment rises to about 45°C, and the humidity is kept high (Table 2), although the

Table 2. Occurrence of zygomycetous species during meju manufacturing

Farm	Manufacturing process		Occurrence of zygomycota				Sales
			From in-process meju		From finished meju		
	LTF (drying)[a] (places, temp., days)	HTF[b] (temp., days)	LTF	HTF	Direct isolation	Dilution plate	
78	out door, -5~10°C, ca. 60 d	ca. 38°C, 15 d	ND[c]	ND	No[d]	Mci[e]	e-shopping
79	glass house, 0~15(20)°C, ca. 45 d	ca. 30°C, 15 d	Mci, Mhi, Mra	Mra, Rpu	No	Lra	e-shopping
80	Vinyl house, -5~10°C, ca. 60 d	ca. 40°C, 15 d	Mci, Mhi, Mmu, Mra	Lor, Lra, Rpu, Rst	Lco, Lra	Lco, Lor, Mci, Rst	e-shopping
98	outdoor, -5~10°C, ca. 75 d	ca. 25°C, 14 d	Mci, Mmu, Mra, Rde	Mci, Mra, Ror	ND	ND	e-shopping
87	Vinyl house, -5~10°C, ca. 60 d	ca. 25°C, 14 d	Mmu, Mra	ND	Mmu, Mra	Lra, Mci, Mmu, Mra, Sra	e-shopping
88	Indoor, 5~20°C, ca. 50 d	No process	Mmu, Mra	ND	Mra, Rst	Lra, Mci	e-shopping
81	No process	ca. 42°C, 21 d	ND	Lra, Rpu	Lra, Rpu	Lra	e-shopping
82	No process (Drying machine, 45°C, 3 d)	ca. 25°C, 21 d	ND	Lra, Mci, Mhi, Rde, Ror	Lra	Lra	e-shopping
84	No process	ca. 35°C, 30 d	ND	Lor, Mci	Lor, Mci	Lor, Mci	e-shopping
89	No process (Drying machine, 45°C, 3 d)	ca. 30°C, 21 d	ND	Rst	Lra, Rde, Rst	Lra, Ror	e-shopping
92	unknown	unknown	ND	ND	No	No	big-box retailer
93	unknown	unknown	ND	ND	Lra	Rpu, Rst	big-box retailer
86	No process	37°C, 30 d	ND	ND	Rde	No	big-box retailer
94	unknown	unknown	ND	ND	Rst	Mci	Traditional market
95	unknown	unknown	ND	ND	Mra, Mlu	Mci, Mlu, Mra	Traditional market
97	unknown	unknown	ND	ND	Mra	Mci, Mra	Traditional market

[a] LTF, Low temperature fermentation process for meju, also referred to as the drying process.
[b] HTF, High temperature fermentation process for meju.
[c] ND, Not determined.
[d] No, No zygomycetous species found.
[e] Abbreviation of species names: Mci, *Mucor circinelloides*; Mhi, *M. hiemalis*; Mmu, *M. mucedo*; Mra, *M. racemosus*; Lor, *Lichtheimia ornata*; Lra, *L. ramosa*; Rpu, *Rhizomucor pusillus*; Rde, *Rhizopus delemar*; Ror, *R. oryzae*; Sra, *Syncephalastrum ramosum.*

conditions depend on the farms. During this period, *Lichtheimia, Mucor, Rhizomucor*, and *Rhizopus* species were observed and *L. ramosa, M. circinelloides*, and *Rhizomucor pusillus*, which can grow at 37°C, were predominant (Table 2). They grew widely on the surface and sometimes invaded the inner parts of meju (Figs. 3D and F).

Currently, some farms (e.g. farms 82 and 89, Table 2) dry meju in drying machines instead of using the LTF. Other farms (e.g. farms 81, 84, and 86, Table 2) go directly to HTF without a drying process. *Mucor* species were only rarely isolated from finished meju products processed without LTF, indicating that LTF stimulates growth of *Mucor* (Table 2).

Unfortunately, the roles of *Mucor* species in meju are not clear. Lee *et al.* (1995) reported that zygomycota species played an important role in making ganjang, and *M. hiemalis, M. circinelloides* and *L. corymbifera* could be used for making delicious ganjang. Lipase production by *M. circinelloides* (Sung, 1965; Hideko *et al.*, 1975) and proteinase, lipase and polyphenoloxidase by *M. racemosus* (Domsch *et al.*, 1993) have been reported. The two species are known to grow under anaerobic conditions (Wood-Baker, 1955; Hesseltine *et al.*, 1985), which means that the species could grow during liquid fermentation under brine. *M. circinelloides* is also known as a mycotoxin decomposer (Detroy and Hesseltine, 1969). More research on the role of *Mucor* in meju production is needed to determine whether the LTH is an essential step for meju fermentation.

The Zygomycota composition differed among meju loaves depending upon where they were purchased. *Mucor* species were the main zygomycota in meju from traditional markets, but mainly *Lichtheimia* and *Rhizomucor* were isolated for meju from big-box discount retailers (Table 2). This difference could be explained by the fact that meju from traditional markets were fermented mainly at low temperatures, while meju from discount retailers were fermented mainly at high temperatures during the entire process. In order to design specific tastes of doenjang and ganjang, the roles of the fungi in meju need to be elucidated.

In this study, 15 species of zygomycota were identified from Korean traditional meju. Some of these species have been reported as being involved in human mycoses for immunocompromised patients, but their pathogenicity is weak, opportunistic, or questionable (Hoog *et al.*, 2000). Furthermore, people do not eat meju, but rather eat ganjang (soy sauce) and doenjang (soybean paste) that are made from meju after more than 2 months of fermentation in brine. Zygomycota has rarely been isolated from ganjang and doenjang, and it is generally considered that zygomycota in the ganjang/doenjang industry is not a problem from a clinical point of view.

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