Mycoflora of Soybeans Used for Meju Fermentation

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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 (excluding Eurotium) (20%), were mainly isolated, and Eurotium herbatorum (22%), Eurotium repens (18%), Cladosporium tenuissimum (18%), F. fujikuroi (18%), Aspergillus oryzae/llavus (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 herbatorum (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 herbatorum, 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 (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, Diaportha, 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.

MATERIALS AND METHODS

From November 2011 to February 2012, we collected
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 plated (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 soybean samples, and 37% of soybean samples were isolated from quite a few factories. 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 enumeration 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%), *Cheatium* (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%), *Paeclomyces* sp. (7.6%), *A. oryzae/fluavus* (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*, *Cheatium*, *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/fluavus*, *A. westerdijkiae*, and *A. versicolor* were the major species isolated from soybeans (Table 2). Of these, *A. oryzae/fluavus* 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 1. Information of the soybeans used in this study](image)

| No. | Location          | Cultivar       |
|-----|------------------|----------------|
| 1   | Chungnam, Gongju | Taekwangkong   |
| 2   | Gyeongbuk, Chilgok | Unknown       |
| 3   | Gyeongg, Ansung  | Daewonkong     |
| 4   | Gyeongg, Ichon   | Daewonkong     |
| 5   | Gyeongg, Yangpyeong | Taekwangkong |
| 6   | Gyeongg, Yongin  | Taekwangkong   |
| 7   | Jeonbuk, Sunchang D | Taekwangkong |
| 8   | Jeonbuk, Sunchang H | Taekwangkong |
| 9   | Jeonnam, Gangiin | Taekwangkong   |
| 10  | Jeonnam, Haenam  | Taekwangkong   |
Table 2. List of fungal species from soybeans and their isolation frequencies

| Scientific name | Representative strains | Incidence on meju<sup>a</sup> | Isolation frequency from sodium hypochlorite untreated soybeans<sup>b</sup> | Isolation frequency from sodium hypochlorite untreated soybeans<sup>c</sup> |
|-----------------|-------------------------|-------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
|                 |                         | Strain No. | Sequence No.<sup>a</sup> | No. of factories<sup>b</sup> | DRBC | DG18 | MEA | No. of factories<sup>c</sup> | DRBC | DG18 | MEA |
| Acremonium      |                         | KACC 47123 | RDA0041296               | 1                          | -    | -    | -   | -                          | -    | -    | -    |
| A. sp.          |                         |            |                            | 1                          | 1    | -    | -   | 1                          | -    | -    | -    |
| Alternaria      |                         | KACC 47124 | RDA0041297               | 7                          | 17   | 1    | 8   | 6                          | 4    | 5    | 7    |
| A. phaeospermum |                         | KACC 47125 | RDA0041298               | 1                          | 17   | 7    | 4   | 2                          | 3    | 1    | 1    |
| Arthrinium      |                         | KACC 47126 | RDA0041299               | 1                          | 5    | -    | -   | -                          | -    | -    | -    |
| A. aculeatus    |                         | KACC 47127 | RDA0041333               | 1                          | -    | -    | 1   | -                          | -    | -    | -    |
| A. creber       |                         | KACC 47128 | RDA0041334               | -                          | -    | -    | -   | 1                          | 2    | -    | -    |
| A. fumigatus    |                         | KACC 47129 | RDA0041335               | **                         | 1    | 1    | -   | -                          | -    | -    | -    |
| A. jenseni      |                         | 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    | -    |
| 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               | **                         | 4    | 2    | 3   | 1                          | 1    | 1    | -    |
| A. versicolor   |                         | KACC 47138 | RDA0041343               | **                         | 4    | 27   | 10  | 4                          | 3    | 2    | 1    |
| A. westerdijkiae|                         | KACC 47139 | RDA0041344               | *                          | 3    | 31   | 15  | 13                         | 1    | -    | 2    |
| Aspergillus sp. |                         |            |                            | 5                          | 17   | 9    | 6   | 3                          | 1    | 4    | -    |
| Bionectria      |                         | KACC 47151 | RDA0041300               | 1                          | 1    | -    | 1   | -                          | -    | -    | -    |
| B. echinoloe    |                         |            |                            | 1                          | 1    | -    | 1   | -                          | -    | -    | -    |
| Biscogniauxia   |                         | KACC 47152 | RDA0041301               | -                          | -    | -    | -   | 1                          | 1    | -    | -    |
| B. mediterranea |                         |            |                            | -                          | -    | -    | -   | 1                          | 1    | -    | -    |
| Botryosphaeria  |                         | KACC 47153 | RDA0041302               | 1                          | -    | 1    | -   | 3                          | 5    | 1    | 3    |
| B. dothidea     |                         |            |                            | 1                          | -    | 1    | -   | 3                          | 5    | 1    | 3    |
| Botrytis sp.    |                         | KACC 47154 | RDA0041303               | *                          | 4    | 5    | -   | 2                          | 3    | 1    | 1    |
| Cercoaspera     |                         | KACC 47155 | RDA0041304               | 2                          | 3    | -    | -   | -                          | -    | -    | -    |
| C. globosum     |                         | KACC 47156 | RDA0041305               | 2                          | 2    | -    | 2   | 3                          | 1    | 1    | 1    |

<sup>a</sup> Incidence on meju: DRBC, DG18, MEA in soybeans.  
<sup>b</sup> Isolation frequency from sodium hypochlorite untreated soybeans.  
<sup>c</sup> Isolation frequency from sodium hypochlorite untreated soybeans.
| Scientific name          | Representative strains | Incidence on meju | Isolation frequency from sodium hypochlorite untreated soybeans | Isolation frequency from sodium hypochlorite untreated soybeans |
|--------------------------|------------------------|-------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
|                          |                        |                   | No. of factories | DRBC | DG18 | MEA | No. of factories | DRBC | DG18 | MEA |
| **Cladosporium**         |                        |                   |                  |      |      |     |                  |      |      |     |
| C. cladosporioides       | KACC 47158 RDA0041369  | **                | 7                | 273  | 189  | 98  | 7                | 27   | 51   | 22  |
| C. halotolerans          | KACC 47159 RDA0041370  | -                 | 1                | 45   | -    | -   | 1                | 1    | 9    | -   |
| 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**         |                        |                   |                  |      |      |     |                  |      |      |     |
| Cochliobolus sp.         | KACC 47163 RDA0041306  | -                 | 1                | -    | -    | -   | 3                | 1    | 8    | 3   |
| **Colletotrichum**       |                        |                   |                  |      |      |     |                  |      |      |     |
| Colletotrichum sp.       | KACC 47164 RDA0041307  | 1                 | 1                | -    | 1    | -   | 2                | 3    | -    | -   |
| **Corynespora**          |                        |                   |                  |      |      |     |                  |      |      |     |
| C. cassicola             | KACC 47165 RDA0041308  | 1                 | 1                | -    | -    | -   | -                | -    | -    | -   |
| **Epicoccum**            |                        |                   |                  |      |      |     |                  |      |      |     |
| E. nigrum                | KACC 47166 RDA0041309  | **                | 1                | 1    | -    | -   | 1                | -    | -    | -   |
| Epicoccum sp.            | KACC 47167 RDA0041310  | -                 | 2                | 4    | 1    | -   | -                | -    | -    | -   |
| **Eurotium (Aspergillus section Aspergillus)** | | | | | | | | | |
| Aspergillus cibarrius    | KACC 47141 RDA0041346  | *                 | 9                | (39) | 254  | (15)| 9                | (38) | 153  | (24)|
| E. anasteladami         | KACC 47142 RDA0041347  | **                | 1                | -    | 1    | -   | -                | -    | -    | -   |
| E. chevalieri           | KACC 47143 RDA0041348  | **                | 3                | -    | 16   | -   | -                | -    | -    | -   |
| E. echinulatum          | KACC 47144 RDA0041349  | **                | 3                | -    | 4    | -   | -                | -    | -    | -   |
| E. herbicariorum        | KACC 47145 RDA0041350  | **                | 8                | -    | 112  | -   | 6                | (14) | 106  | (1) |
| E. manginii             | KACC 47146 RDA0041351  | 2                 | -                | 42   | -    | 1   | 1                | (1)  | 7    | (2) |
| E. niveoglaucum/medium   | KACC 47147 RDA0041352  | 2                 | -                | 6    | -    | 1   | -                | 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**            |                        |                   |                  |      |      |     |                  |      |      |     |
| E. andiyazi             | KACC 47168 RDA0041374  | -                 | 10               | 164  | 45   | 141| 8                | 11   | 16   | 4   |
| E. concentricum         | KACC 47169 RDA0041375  | 2                 | 1                | 1    | 1    | -   | 1                | 1    | -    | -   |
| E. fujikuroi (anamorph of Gibberella fujikuroi) | KACC 47170 RDA0041376  | *                 | 5                | 92   | 14   | 97 | 3                | 3    | 1    | 1   |
| E. cf. incarnatum       | KACC 47171 RDA0041377  | *                 | 2                | 2    | 9    | 1   | 2                | 3    | 2    | 2   |
| E. oxyosphorum          | KACC 47172 RDA0041378  | 2                 | 1                | 1    | -    | -   | 2                | 1    | 1    | -   |
| E. proliferatum         | KACC 47173 RDA0041379  | 2                 | 7                | 15   | 4    | -   | -                | -    | -    | -   |
| E. solani               | KACC 47174 RDA0041380  | 1                 | 5                | 3    | 2    | -   | 1                | 1    | -    | -   |
| Fusarium sp.            |                        |                   | 5                | 61   | 4    | 37 | 3                | 3    | 11   | -   |
| Scientific name            | Representative strains | Incidence on meju | Isolation frequency from sodium hypochlorite untreated soybeans | Isolation frequency from sodium hypochlorite untreated soybeans |
|---------------------------|------------------------|-------------------|---------------------------------------------------------------|---------------------------------------------------------------|
|                           |                        |                   | No. of factories | DRBC | DG18 | MEA | No. of factories | DRBC | DG18 | MEA |
| Irpex                     |                        |                   |                  |      |      |     |                  |      |      |     |
| I. lacteus                | KACC 47175 RDA0041311  | 1                 | 4               | 3    | 1    | -   | -                | -    | -    | -   |
| Khuska                    |                        |                   | 1               | 2    | -    | 6   | 1                | -    | -    | 3   |
| K. oryzae                 | KACC 47176 RDA0041312  | -                 | -               | -    | -    | -   | 1                | -    | -    | 3   |
| Khuska 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               | -    | -    | -   | -                | -    | -    | -   |
| 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. cecidica               | 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                | 1    | 1    | -   |
| 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    | 1    | -   |
| P. steckii                | KACC 47198 RDA0041366  | *                | 5               | 32   | 12   | 22  | 1                | 1    | 1    | -   |
| P. sumatrense             | KACC 47199 RDA0041367  |                   | 1               | -    | 5    | -   | -                | -    | -    | -   |
| P. toxicariurn            | KACC 47200 RDA0041368  |                   | -               | -    | -    | -   | 1                | 1    | -    | -   |
| Penicillium sp.           |                        |                   | 6               | 28   | 9    | 17  | 2                | 1    | 22   | -   |
| Scientific name     | Representative strains | Incidence on meju<sup>b</sup> | Isolation frequency from sodium hypochlorite untreated soybeans<sup>c</sup> | Isolation frequency from sodium hypochlorite untreated soybeans<sup>c</sup> |
|---------------------|-------------------------|-------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
|                     |                         | Strain No.                  | Sequence No.                | No. of factories<sup>d</sup> | DRBC | DG18 | MEA | No. of factories<sup>d</sup> | DRBC | DG18 | MEA |
| Pestalotiopsis      | KACC 47201 RDA0041319   | 1                            | -                           | 1                           | -    | -    | 1   | 1                           | -    | -    | 1   |
| Pestalotiopsis sp.  | KACC 47202 RDA0041320   | 1                            | -                           | 1                           | -    | -    | 1   | 1                           | -    | -    | 1   |
| Phoma               |                         | 5                            | 14                          | 1                            | 8    | 3    | 3   | 1                           | -    | -    | -   |
| P. herbarum         | KACC 47203 RDA0041321   | 5                            | 7                           | 1                            | 3    | 2    | 2   | 1                           | -    | -    | -   |
| Phomopsis           |                         | 4                            | 5                           | 1                            | 17   | 4    | 2   | 1                            | 3    | 3    | 1   |
| P. longicolla       | KACC 47204 RDA0041322   | 4                            | 5                           | 1                            | 15   | 1    | 1   | 1                           | -    | -    | -   |
| Phomopsis sp.       | KACC 47205 RDA0041323   | 3                            | 15                          | 9                            | 15   | -    | -   | -                           | -    | -    | -   |
| Rhizopus            | KACC 47206 RDA0041324   | 3                            | 1                           | 1                            | -    | -    | -   | -                           | -    | -    | -   |
| R. microsporus      | KACC 47207             | 1                            | 1                           | -                            | -    | -    | -   | -                           | -    | -    | -   |
| Phomopsis sp.       | KACC 47208 RDA0041325   | 1                            | 1                           | -                            | -    | -    | -   | -                           | -    | -    | -   |
| Schizophyllum        | KACC 47209 RDA0041326   | -                            | -                           | -                            | -    | -    | -   | 1                           | 1    | 2    | 1   |
| S. commune          | KACC 47210 RDA0041327   | -                            | -                           | -                            | -    | -    | -   | 1                           | -    | -    | -   |
| Scopulariopsis      | KACC 47211 RDA0041328   | -                            | -                           | -                            | -    | -    | -   | 1                           | 1    | 2    | 2   |
| Sphaerodes          | KACC 47212 RDA0041329   | *                            | 1                           | -                            | 1    | -    | -   | -                           | -    | -    | -   |
| Sphaerodes sp.      | KACC 47213 RDA0041330   | 1                            | -                           | -                            | 2    | -    | -   | -                           | -    | -    | -   |
| Stemphylium         | KACC 47214             | 1                            | 1                           | -                            | -    | -    | -   | -                           | -    | -    | -   |
| Stemphylium sp.     | KACC 47215 RDA0041331   | 1                            | 3                           | -                            | -    | -    | -   | -                           | -    | -    | -   |
| Syncephalastrum     | KACC 47216 RDA0041332   | -                            | -                           | -                            | 1    | 1    | -   | -                           | -    | -    | -   |

<sup>a</sup>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).
<sup>b</sup>The species were isolated from meju, with ***high frequency, **medium frequency, or *low frequency.
<sup>c</sup>The frequency indicates the number of soybeans from which the species were isolated from 500 soybeans.
<sup>d</sup>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. harbiorum*, *E. repens* and *E. mangini* 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. pseudoocladosporioideae*, and *C. cladosporioideae* were mainly isolated from soybeans, whereas *C. tenuissimum* and *C. sphacerospermum* 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 *zygomyctota* cannot utilize its nutrients. Therefore, *zygomyctota* 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*, *Khukia 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 cladosporioideae*, *Eupenicillium cinnamopurpureum*, *Eurotium amstelodami*, *Eurotium restrictum*, *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*, *Khukia*, *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.](image-url)
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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