Isolation and Characterization of Three Zygomycetous Fungi in Korea: Backusella circina, Circinella muscae, and Mucor ramosissimus

Thuong T. T. Nguyen and Hyang Burm Lee

Division of Food Technology, Biotechnology & Agrochemistry, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, South Korea

ABSTRACT
While surveying undiscovered fungal taxa in Korea, three rare zygomycetous fungal strains, CNUFC-PTF2-1, CNUFC-TF3-1, and CNUFC-ESAF3-1, were isolated from soil, leaf, and freshwater samples, respectively. The strains were analyzed morphologically as well as phylogenetically based on the internal transcribed spacer region and 28S rDNA sequences. Sequence analysis of the two loci revealed that the isolates, CNUFC-PTF2-1, CNUFC-TF3-1, and CNUFC-ESAF3-1, were identified as Backusella circina, Circinella muscae, and Mucor ramosissimus, respectively. These species have not yet been previously described in Korea.

1. Introduction
The Mucorales, which is classified into the subphylum Mucoromycotina [1], is the largest order of fungi. Members of this group are ubiquitous saprophytes in nature. They are commonly found in soil and decaying vegetation, and can also be found in grains [2–4]. Mucorales members grow and invade quickly on easily digestible substrates, such as those containing starches, sugars, and hemicelluloses [2]. Currently, 14 families are placed in this order based on the analysis of a multigene (act-1, EF-1a, 18S, and 28S rRNA) data set [3].

The genus Backusella, which belongs to the subphylum Mucoromycotina, order Mucorales, and family Backusellaceae, was established by Ellis & Heseltine (1969) with the type species B. circina [5]. Species belonging to this genus are characterized by the production of both sporangia and sporangiophores, while C. muscae was transferred to the genus Mucor. Based on these criteria, this genus now comprises 13 species, among which one species is registered in Korea (Source: www.indexfungorum.org as of April 2018).

The genus Circinella, which belongs to the subphylum Mucoromycotina, order Mucorales, and family Lichtheimiaceae, was established in 1873 by van Tieghem and Le Monnier [11]. It is closely related to Mucor, but differs in that it has sporangiophores with circinate branches bearing sporangia [12]. Members of this genus are characterized by the production of sporangiophores bearing circinate branches terminated by globose multispored sporangia with persistent sporangial walls [12]. After Heseltine and Fennell [12] monographed this genus, several additional species were included [13–16]. To date, 10 species belonging to this genus are known according to the Index Fungorum (www.indexfungorum.org). They are commonly isolated from soil, dung, sand beach, and hydrocarbon-polluted sand [2,17,18].

The genus Mucor belongs to the subphylum Mucoromycotina, order Mucorales, and family Mucoraceae, and was described by Fresenius in 1850, comprising the largest number of species within the Mucorales [19]. Specimens of this genus are characterized by the formation of non-apophysate sporangia and production of simple or branched sporangiophores without basal rhizoids. Zygospores have opposed, non-appendaged suspensors [20]. Mucor species are easily isolated from soil, fruits, vegetables, stored grains, insect, and dung [2,21–24]. Several species of this genus are of great interest to the
biotechnology industry due to their ability to produce enzymes such as proteases, amylase, lipases, phytase, and polygalacturonase [25–27], while some species are considered as the causal agents of cutaneous zygomycosis in humans [28]. The traditional taxonomic classification of *Mucor* species was determined based on morphological characteristics such as size and shape of the sporangia and mode of reproduction (sexual or asexual). Recently, molecular studies have been performed to evaluate mucoralean species [3,4]. These studies have indicated that *Mucor* is polyphyletic. Based on the phylogenetic analysis of ITS and large subunit rDNA regions of several mucoralean species, Walther et al. [4] observed that some *Mucor* species with curved sporangiophores were grouped with *Backusella* Hesselt. & J. J. Ellis. Therefore, these *Mucor* species were transferred to *Backusella*. Nine species have been recorded, including three new species from freshwater, tangerine fruit, and rat feces samples in Korea [10,23].

During an inventory of fungal species from soil, leaf, and freshwater samples, three interesting fungal strains belonging to the order Mucorales were assigned to the genera *Backusella*, *Circinella*, and *Mucor*.

The objective of this study was to morphologically and molecularly characterize three unrecorded species in Korea: *B. circina*, *C. muscae*, and *M. ramosissimus*.

2. Materials and methods

2.1. Isolation of fungal strains from leaf, soil, and freshwater samples

Leaves of *Toxicodendron sylvestre* were collected from Daegak Mountain, Sinsi Island, Gunsan, Korea. Collected samples were stored in sterile polyethylene bags. Samples were cleaned under running tap water to remove debris before use. Leaf tissue pieces were cut into small fragments, surface-disinfested with 2% NaOCl solution and 70% ethanol for 1 min each, washed three times with sterile distilled water, plated on potato dextrose agar (PDA; BD Biosciences, Franklin Lakes, NJ) supplemented with the antibiotic streptomycin sulfate (0.5 mg/mL, Sigma-Aldrich, St. Louis, MO), and incubated at 25°C for 3–7 days. To isolate pure cultures, individual colonies with various morphologies were picked, transferred to PDA, and subcultured until pure mycelia were obtained. All pure isolates, including those of *B. circina*, *C. muscae*, and *M. ramosissimus*, were stored in 20% glycerol at −80°C at the Chonnam National University Fungal Collection (CNUFC), Gwangju, Korea. *B. circina*, *C. muscae*, and *M. ramosissimus* strains isolated in our study were designated CNUFC-PTF2-1 and CNUFC-PTF2-2, CNUFC-TF3-1 and CNUFC-TF3-2, CNUFC-ESAF3-1 and CNUFC-ESAF3-2, respectively. Strain CNUFC-PTF2-1 was also deposited at the Culture Collection of the National Institute of Biological Resources (NIBR, Incheon, Korea), strain CNUFC-TF3-1 was deposited at Korean Agricultural Culture Collection (Wanju, Korea), and strain CNUFC-ESAF3-1 was deposited at the Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR, Sangju, Korea).

2.2. Morphological studies

Pure cultures of *B. circina*, *C. muscae*, and *M. ramosissimus* were cultured on synthetic mucor agar (SMA; 40 g dextrose, 2 g asparagine, 0.5 g KH2PO4, 0.25 g MgSO4.7H2O, 0.5 g thiamine chloride, and 15 g agar in 1 L of deionized water). The plates were incubated at 10, 15, 20, 25, 30, 35, and 40°C in the dark for 5 days. Fragments of mycelia were removed from the cultures, placed on microscope slides with lactophenol solution (Junsei Chemical Co. Ltd., Tokyo, Japan) and observed under a light microscope (Olympus, Tokyo, Japan). The fine structure of *C. muscae* was observed using scanning electron microscopy (Hitachi S4700; Hitachi, Tokyo, Japan). The isolates were fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 2 h and then washed with 0.05 M cacodylate buffer (Junsei Chemical Co. Ltd.). Cellular membranes were preserved by fixing the samples in 1% osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA) diluted in 0.05 M cacodylate buffer for 1 h, washing again in 0.05 M cacodylate buffer, dehydrating in graded ethanol (Em sure, Darmstadt, Germany) and isoamyl acetate (Junsei Chemical Co. Ltd.), and drying in a fume hood. Finally, the samples were sputter-coated with gold and observed under a Hitachi S4700 field emission scanning electron microscope at the Korea Basic Science Institute (Gwangju, Korea).

2.3. DNA extraction, PCR, and sequencing

Genomic DNA was extracted directly from the mycelia of fungal isolates using the Solgent
**Table 1.** Taxa, collection numbers, sequences, and GenBank accession numbers used in this study.  

| Taxon name | Collection no. (Isolate no.) | ITS | 28S |
|------------|-------------------------------|-----|-----|
| Backusella circina | CBS 128.70<sup>T</sup> | JN206258 | JN206529 |
| B. circina | KHI | JK644544 | JK646449 |
| B. circina | CBS 323.69 | JN206269 | – |
| B. circina | KHI | – | JX644492 |
| B. circina | CNUFC-PTF2-1 | MH262302 | MH262312 |
| B. circina | CNUFC-PTF2-2 | MH262303 | MH262313 |
| B. constricta | URM7322 | KF731759 | KF731758 |
| B. indica | CBS 786.70 | JN206255 | JN206526 |
| B. giganteus | CCIB 8466 | KF742415 | – |
| B. grandis | CBS 186.87<sup>T</sup> | JN206252 | JN206527 |
| B. lamprosora | CBS 118.08<sup>T</sup> | JN206268 | JN206531 |
| B. lamprosora | CBS 195.28 | JN206271 | JN206530 |
| B. locutae | CNUFC-SFB2<sup>T</sup> | KY449291 | KY449290 |
| B. locutae | CNUFC-SFB4 | KI449293 | KY449292 |
| B. oblongiopora | CBS 568.70 | JN206278 | JN206533 |
| B. recurva | KH6 | – | JX644497 |
| B. recurva | KH7 | – | JX644498 |
| B. recurva | CBS 318.52 | JN206261 | – |
| B. recurva | CBS 673.75 | JN206264 | – |
| B. tuberculospora | CBS 562.66 | JN206267 | JN206525 |
| B. tuberculospora | CBS 570.70 | JN206266 | – |
| B. variabilis | CBS 564.66 | JN206254 | KCO12658 |
| B. variabilis | CBS 564.66 | JN206253 | – |
| Circinella angaransis | CBS 173.62 | JN205849 | JN206551 |
| C. chinensis | CBS 140.28 | JN205853 | JN206549 |
| C. lusitanicus | CBS 101757 | JN206409 | JN206550 |
| C. minor | CBS 142.81 | JN205861 | JN206552 |
| C. mucoroides | CYD1000719 | KF805760 | KF805746 |
| C. muscae | CCD1000215 | – | KF805745 |
| C. muscae | CBS 141.28 | JN205853 | JN206548 |
| C. muscae | CBS 107.13 | JN205854 | – |
| C. muscae | CYR003 | – | KF805748 |
| C. muscae | D001229001 | KF805764 | KF805750 |
| C. muscae | D00122901 | KF805765 | – |
| C. muscae | D00122902 | KF805766 | – |
| M. amphibiorum | NRRL28633 | – | AF113466 |
| M. circinelloides | CBS 338.71 | JN205998 | – |
| M. circinelloides | CBS 635.65 | JN205997 | – |
| M. circinelloides | UTHSC 04-1961 | – | FN506567 |
| M. circinelloides | – | – | FN506566 |
| M. circinelloides f. janssenii | CBS 232.29 | JN206007 | – |
| M. circinelloides f. janssenii | CBS 206.68 | JN206004 | – |
| M. circinelloides f. janssenii | CBS 205.68<sup>NT</sup> | HM999952 | – |
| M. circinelloides f. janssenii | CBS 526.68 | – | JN206426 |
| M. circinelloides f. circinelloides | CBS 195.68 | – | NG_055735 |
| M. circinelloides f. circinelloides | Kw1378 | – | FM246460 |
| M. circinelloides f. lusitanicus | CBS 108.17 | JN205980 | FN506565 |
| M. circinelloides f. lusitanicus | CBS 851.71 | JN205982 | – |
| M. circinelloides f. lusitanicus | CBS 111228 | JN205989 | – |
| M. circinelloides f. lusitanicus | CBS 242.33 | JN205987 | – |
| M. circinelloides f. lusitanicus | CBS 968.68 | HM999953 | – |
| M. circinelloides f. lusitanicus | UTHSC 03-1823 | – | FN506562 |
| M. circinelloides f. lusitanicus | – | – | AF113467 |
| M. circinelloides f. lusitanicus | – | – | – |
| M. fragilis | CTSP F1 | EU862184 | EU862173 |
| M. fragilis | FSU 6164 | EU484238 | – |
| M. plumbeus | CBS 634.74 | HM999955 | – |
| M. plumbeus | CBS 226.32 | JN205916 | – |
| M. indicus | CBS 226.29 | HM999956 | HM849690 |
| M. sinensis | CBS 204.74 | JN205899 | – |
| M. racemosus | – | UWP 788 | AY213712 |
| M. racemosa f. chibnensis | CBS 636.67 | JN205904 | – |
| M. racemosa f. racemosa | CBS 260.68<sup>NT</sup> | – | NG_055727 |
| M. ramossissimus | CBS 135.65<sup>NT</sup> | NR_103627 | FN506566 |
| M. ramossissimus | – | ATCC 28933 | AY213715 |
| M. ramossissimus | – | CNUFC-EASF3-1 | MH262306 |
| M. ramossissimus | – | CNUFC-EASF3-2 | MH262307 |
| Phascolomyces articulosus | CBS 113.76 | JX65309 | JN206547 |
| Rhizomucor pusillus | CBS 354.68 | AF417646 | HM849756 |
| Zychae mexicana | CBS 441.76 | JN205845 | JN206546 |

Bold letters indicate isolates and accession numbers determined in our study.

ATCC: American Type Culture Collection, Manassas, VA, USA; CBS: Centraalbureau voor Schimmecultures, Utrecht, The Netherlands; CNUFC: Chonnam National University Fungal Collection, Gwangju, South Korea; ITS: internal transcribed spacer; NRRL (ARS Culture Collection, Peoria, Illinois); UTHSC, Fungal Testing Laboratory, Department of Pathology at the University of Texas Health Science Center, San Antonio, Texas, USA; <sup>1</sup> and <sup>2</sup>: ex-type and ex-neotype strains.
Genomic DNA prep Kit (Solgent Co. Ltd., Daejeon, South Korea). The ITS region and large subunit of 28S rDNA were amplified with the primer pairs ITS4 and ITS5 [29] and LROR and LR5F [30], respectively. The PCR mixture (total volume, 20 μL) contained fungal DNA template, 5 pmol/μL of each primer, and Accupower PCR Premix (Taq DNA polymerase, dNTPs, buffer, and tracking dye; Bioneer Corp., Daejeon, Korea). PCR products were purified using the Accuprep PCR Purification Kit (Bioneer Corp.) according to the manufacturer’s instructions. DNA sequencing was performed on an ABI 3700 Automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA).

2.4. Phylogenetic analysis

The fungal sequences obtained from the GenBank database (Table 1) were aligned using Clustal_X v.1.83 [31] and edited with Bioedit v.5.0.9.1 [32]. Phylogenetic analyses were performed using MEGA 6 software [33] and maximum likelihood (ML) was constructed by Kimura’s two-parameter correction method. *M. amphibiorum, M. indicus,* and *Rhizomucor pusillus* were used as outgroups. The reliability of internal branches was assessed using the p-distance substitution model with 1000 bootstrap replicates. Sequence data were compared with similar sequences available in the GenBank databases using nucleotide Basic Local Alignment Search Tool (BLASTn).

3. Results

3.1. Phylogenetic analysis

A BLAST search of ITS sequences via the NCBI database indicated that the isolates CNUFC-PTF2-1, CNUFC-TF3-1, and CNUFC-ESAF3-1 most closely
resembled *B. circina* (GenBank accession no. JX644544), *C. muscae* (GenBank accession no. KF805764), and *M. ramosissimus* (GenBank accession no. NR_103627) with 100% (637/637 bp), 99.8% (601/602 bp), and 99.8% (566/567 bp) homology, respectively. The 28S rDNA sequences of *B. circina* (GenBank accession no. JN206529), *C. muscae* (GenBank accession no. KF805750), and *M. ramosissimus* (GenBank accession no. FN650666) showed 100% (674/674 bp), 98.9% (550/556 bp), and 100% (694/694 bp) homology with the 28S rDNA sequences of the isolates CNUFC-PTF2-1, CNUFC-TF3-1, and CNUFC-ESAF3-1, respectively. Based on the ITS and 28S rDNA trees, the isolates CNUFC-PTF2-1, CNUFC-TF3-1, and CNUFC-ESAF3-1 were identical to *B. circina*, *C. muscae*, and *M. ramosissimus*, respectively (Figures 1–3).

### 3.2. Taxonomy

#### 3.2.1. Taxonomy of CNUFC-PTF2-1

**Backusella circina** J.J. Ellis & Hesselt., Mycologia 61: 865 (1969) (Table 2, Figure 4).

*Backusella circina* is identical to *Mucor pseudolamprosporus* H. Nagan. & Hirahara, Hiroshima Jogakuin College Bull.: 167 (1968).

**Description:** Colonies grew rapidly at 25°C on SMA, filling the Petri plate (diameter, 90 mm) after 5 days of incubation. The colonies were initially white, but later turned light gray. The colony reverse was light gray. Sporangiophores were 6–20 μm wide, erect, branched, and irregular. Sporangia were globose to subglobose, multispored, and measured 27.5–66.5 μm. Columellae were subglobose to oblong, and measured 18.1–30.4 μm × 20.3–34.6 μm. Unispored sporangiola were abundant, globose to subglobose, wall spinulose, and measured 10.3–19.5 μm × 10.0–19.1 μm. Sporangiospores were subglobose to ovoid, and measured 6.5–11.0 μm × 6.0–10.2 μm. Zygospores were not observed on this medium. Optimal growth was observed at 25°C, slow growth was observed at 10 and 35°C, and no growth was observed at 37°C.

#### 3.2.2. Taxonomy of CNUFC-TF3-1

**Circinella muscae** (Sorokin) Berl. & De Toni, Sylloge Fungorum 7: 216 (1888) (Table 3, Figure 5).

**Description:** Colonies grew rapidly at 25°C on SMA, filling the Petri plate (diameter, 90 mm) after 5 days of incubation. The colonies were initially white, but later turned light gray. The colony reverse was light gray. Sporangiophores were 6–20 μm wide, erect, branched, and irregular. Sporangia were globose to subglobose, multispored, and measured 27.5–66.5 μm. Columellae were subglobose to oblong, and measured 18.1–30.4 μm × 20.3–34.6 μm. Unispored sporangiola were abundant, globose to subglobose, wall spinulose, and measured 10.3–19.5 μm × 10.0–19.1 μm. Sporangiospores were subglobose to ovoid, and measured 6.5–11.0 μm × 6.0–10.2 μm. Zygospores were not observed on this medium. Optimal growth was observed at 25°C, slow growth was observed at 10 and 35°C, and no growth was observed at 37°C.
T. T. T. NGUYEN AND H. B. LEE

Reverse was brown and irregularly zonate. The colony initially white, but later turned brown. The colonies were initiated after 7 days of incubation. The colonies were initially white, but later turned brown. The colony reverse was brown and irregularly zonate.

Table 2. Morphological characteristics of CNUFC-PTF2-1 compared to Backusella circina reference strain.

| Character                  | CNUFC-PTF2-1                                      | Backusella circina* |
|----------------------------|--------------------------------------------------|---------------------|
| Colony color               | Rapid-growing, first white then light gray, reverse light gray | Rapid-growing, first white then light olive-gray |
| Sporangiophores            | 6.0–20.0 µm in width, variable in length          | Up to 9–16 µm in width, variable in length |
| Sporangia                  | Globose to subglobose, multisporous, 27.5–66.5 × 27.0–63.5 µm | Globose and subglobose, 35–100 µm |
| Columellae                 | Subglobose to oblong, 18.1–30.4 × 20.3–34.6 µm    | Subglobose to oblong, 11–35 × 11–30 µm |
| Unisporous sporangiola     | Globose to subglobose 10.3–19.5 × 10.0–19.1 µm     | Globose to subglobose, (4.5–)6–16(–26) µm in diam, wall spinulose |
| Sporangiospores            | Subglobose to ovoid, 6.5–11.0 × 6.0–10.2 µm        | Subglobose to ovoid, (6.4–)7.2–10 (–12.8) × (5.6–)6.4–9.2 (–10) µm |
| Zygospores                 | Absent                                           | Globose to subglobose, (35–)140–70(–80) µm in diam. |

*From the description by Ellis and Hesseltine [5].

=Circinella spinosa= Tiegh. & G. Le Monn., Annales des Sciences Naturelles Botanique 17: 305 (1873)

=Circinella sydowii= Lendn., Bulletin de la Société Botanique de Genève 5: 29 (1913)

Description: Colonies grew rapidly at 25°C on SMA, filling the Petri plate (diameter, 90 mm) after 7 days of incubation. The colonies were initially white, but later turned brown. The colony reverse was brown and irregularly zonate. Sporangiophores were 6.3–10.8 µm in width, variable in length, and often circinate below the sporangium. Sporangia were globose, yellow to dark gray, multisporous, and measured 31.9–70.2 µm × 31.5–69.2 µm. Collumellae were diverse in shape, pyriform, subglobose, oval, conical, and measured 17.5–43.3 µm × 15.5–36.5 µm. Sporangiospores were globose and measured 4.1–9.5 µm. Zygospores were not observed on this medium. Optimal growth was observed at 25°C, slow growth was

Figure 3. Phylogenetic tree based on maximum likelihood analysis of internal transcribed spacers (ITS) and 28S rDNA sequences for M. ramosissimus CNUFC-ESAF3-1 and M. ramosissimus CNUFC-ESAF3-2, Mucor amphibiourum was used as the outgroup. Bootstrap support values ≥50% are indicated at the nodes. The bar indicates the number of substitutions per position.
observed at 10 and 35°C, and no growth was observed at 35°C.

3.2.3. Taxonomy of CNUFC-ESAF3-1

*Mucor ramosissimus* Samouts., Mater. Mikol. Fitopat. Ross.: 210 (1927) (Table 4, Figure 6)

**Description:** Colonies grew rapidly at 25°C on SMA, filling the Petri plate (diameter, 90 mm) after 5 days of incubation. The color of the colony was gray. Sporangia were globose to subglobose and measured 15.5–60.1 μm × 14.8–56.3 μm. Columellae were globose, cylindrical–ellipsoidal, and measured 12.7–41.8 μm × 11.8–36.8 μm. Sporangiospores were subglobose, ellipsoidal, and measured 4.3–8.5 μm × 3.9–6.8 μm. Zygospores were not observed on this medium. Optimal growth was observed at 25°C, slow growth was observed at 10°C, and no growth was observed at 35°C.

4. Discussion

To date, few studies have reported new and undescribed zygomycetous fungi in Korea [10,23,34,35]. Particularly, species of *Backusella* and *Circinella* are rarely found in Korea. Thus, our finding of *B. circina*, *C. muscae*, and *M. ramosissimus* species not only establishes new records, but also provides knowledge regarding the occurrence and distribution of rare species within these genera.

Variability in nucleotide sequences in the ITS region has been reported by several authors as a critical barcode marker for identifying fungi at the species level, including in the order Mucorales [36,37]. In previous studies, we successfully identified species of Mucorales using this marker [10,22,23,38].

In the ITS and 28S phylogenetic trees, CNUFC-PTF2-1 and CNUFC-PTF2-2 isolated from soil samples clustered in the clade containing *B. circina* CBS.
128.70 (type species) (Figure 1). Although the morphological features of our isolate were similar to those of *B. circina* described by Ellis and Hesseltine [5], there were differences in the diameter of sporangia and unispored sporangiola. Sporangia sizes reported in literature range from 35 to 100 μm [5], which are larger than our maximum measurements. The unispored sporangiola (4.5–6–16(–26) μm [5] was larger than that of our isolate. The *B. circina* strain may exhibit morphological similarities to *B. lamprospora*, such as the production of subglobose sporangiospores [7]. However, *B. circina* differs from *B. lamprospora* in its production of large numbers of spiny-walled and unispored sporangia. Furthermore, in the phylogenetec tree, the strain formed a separate branch from that of *B. lamprospora*. Molecular data confirmed the morphological identification of CNUFC-PTF2-1 as *B. circina*.

Isolates CNUFC-TF3-1 and CNUFC-TF3-2 formed a group with strains of *C. muscae* (Figure 2). The results of our analysis of molecular data were consistent with the phylogeny presented by Walther et al. [4]. Comparing the colony morphology and culture characteristics of the isolate on SMA medium with previous descriptions by Hesseltine and Fennell [12], the present isolate was generally similar to isolates of *C. muscae*. Gonzalez et al. [17] and Zheng et al. [18] isolated this species from sand beach and hydrocarbon-polluted sand, respectively. Accordingly, this is the first reported isolation of *C. muscae* from a leaf of *T. sylvestre*. *C. muscae* has been shown to transform androst-4-ene-3,17-dione and produce extracellular enzymes such as proteases [39,40]. This finding suggests that strain CNUFC-TF3-1 may be useful in biotechnological applications and requires further investigation.

**Table 4.** Morphological characteristics of CNUFC-ESAF3-1 compared to *Mucor ramosissimus* reference strain.

| Character       | CNUFC-ESAF3-1 | *Mucor ramosissimus*<sup>a</sup> |
|-----------------|---------------|----------------------------------|
| Colony color    | Rapid-growing, gray | Deep olive gray to mouse gray |
| Sporangia       | Globose to subglobose, 15.5–60.1 × 14.8–56.3 μm | Globose, up to 70 (80) μm |
| Columellae      | Globose, cylindrical–ellipsoidal, 12.7–41.8 × 11.8–36.8 μm | Applanate, up to 40 × 50 μm |
| Sporangiospores | Subglobose, ellipsoidal, 4.3–8.5 × 3.9–6.8 μm | Subglobose to broadly ellipsoidal, 4–7(8) μm in diam. or 5–8 × 4.5–6 μm |
| Zygospores      | Absent        | Absent                           |

<sup>a</sup>From the description by Schipper [21].
Isolate CNUFC-ESAF3-1 was grouped with strains of *M. ramosissimus* CBS 135.65 (neo-type species) based on phylogenetic analyses (Figure 3). The morphological characteristics of the *M. ramosissimus* isolate in this study were similar to those previously described by Schipper [21]. However, sporangia were smaller than that of *M. ramosissimus* described by Schipper (up to 70–80 μm). The *M. ramosissimus* strain may be confused with *M. circinelloides* due to its production of subglobose sporangiospores and sympodially branched sporangiophores. However, there are clear genetic differences between these two species. The results of our analysis of molecular data of this species were consistent with the phylogeny presented by Álvarez et al. [41] and Walther et al. [4]. Species of *M. ramosissimus* produce extracellular enzymes, such as endopolygalacturonase and lipase, and secondary metabolites, such as phytoalexin elicitor [42–44]. Recently, several studies have focused on applying Mucorales members to produce ethanol and biomass by-product [45]. Particularly, *M. ramosissimus* has been reported as a potential ethanol-producing mold [46].

This is the first report of *B. circina*, *C. muscae*, and *M. ramosissimus* in Korea. Future studies should investigate their ability to produce extracellular enzymes and potential applications in biotechnology.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was in part supported by the Graduate Program for the Undiscovered Taxa of Korea, and in part by the Project on Survey and Discovery of Indigenous Fungal Species of Korea funded by NIBR and Project on Discovery of Fungi from Freshwater and Collection of Fungarium funded by NNIBR of the Ministry of Environment (MOE), and in part carried out with the support of Cooperative Research Program for Agriculture Science and Technology Development [PJ013744], Rural Development Administration, Republic of Korea. This work was in part supported by the BK21 plus program through the National Research Foundation (NRF) funded by the Ministry of Education of Korea.

**ORCID**

Hyang Burm Lee http://orcid.org/0000-0001-5353-2586

**References**

[1] Hibbett DS, Binder M, Bischoff JF, et al. A higher-level phylogenetic classification of the fungi. Mycol Res. 2007;111:509–547.
[2] Benny GL. The methods used by Dr. R. K. Benjamin, and other mycologists, to isolate Zygomycetes. Aliso, 2008;26:37–61.

[3] Hoffmann K, Pawłowska J, Walther G, et al. The family structure of the Mucorales: a synoptic revision based on comprehensive multigene-geologicalios. Persoonia. 2013;30:57–76.

[4] Walther G, Pawłowska J, Alastraucy-Izquierdo A, et al. DNA barcoding in Mucorales: an inventory of biodiversity. Persoonia. 2013;30:11–47.

[5] Ellis JJ, Hesselteine CW. Two new members of the Mucorales. Mycologia. 1969;61:863–872.

[6] Kirk PM. Nomenclatural novelties. Index Fungorum. 2013:11–1.

[7] Benny GL, Benjamin RK. Observations on Thamnidiaceae (Mucorales). New taxa, new combinations, and notes on selected species. Aliso. 1975:8:301–351.

[8] de Souza JI, Marano AV, Pires-Zottarelli CLA, et al. A new species of Backussula (Mucorales) from a Cerrado reserve in Southeast Brazil. Mycol Prog. 2014;13:975–980.

[9] Lima DX, Voigt K, de Souza CAF, et al. Description of Backussula constricta sp. nov. (Mucorales, ex Zygomycota) from the Brazilian Atlantic Rainforest, including a key to species of Backussula. Phytotaxa. 2016;289:59–68.

[10] Wanasinghe DN, Phukhansakda C, Hyde KD, et al. Fungal diversity notes 709–839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. Fungal Divers. 2018;91:1–236.

[11] van Tieghem P, Le Monnier G. Recherches sur les Mucor circinelloides. Mucor circinelloides. 1919.

[12] Hesseltine CW, Fennell DI. The genus Circinella (Mucorales). New taxa, new combinations, and notes on selected species. Aliso. 1975:8:301–351.

[13] Hesselteine CW, Fennell DI. The genus Circinella. Mycologia. 1955:47:193–212.

[14] Hesselteine CW, Ellis JI. Notes on Mucorales, especially Absidia. Mycologia. 1961;53:406–426.

[15] Faurel L, Schotter G. Notes mycologiques VI. Sur quelques champignons coprophiles d’Afrique Equatoriale. Cah La Mabok. 1965;3:123–133.

[16] Patil SD, Kale JC. A new species of Circinella van Tiegh. and Le Monn. Curr Sci. 1981;50:544–544.

[17] Arambbari AM, Cabello MN. Circinella lacrymispora sp. nov., a new mucoral isolated from Argentine soils. Mycotaxon. 1996;57:145–149.

[18] González MC, Murueta-Figueroa N, Medina-Ortiz C, et al. New record of Circinella muscae from a hydrocarbon polluted sand beach of Tabasco, Mexico. Mycotaxon. 2010;113:111–117.

[19] Zheng RY, Liu XY, Wang YN. Circinella (Mucorales, Mucoromycotina) from China. Mycotaxon. 2017;132:43–62.

[20] Spatafora JW, Benny GL, Lazarus K, et al. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia. 2016;108:1028–1046.

[21] Benny GL, Humber RA, Voigt K. Zygomyceorous fungi: Phylum Entomophthoromycota and subdivision Kickxellomycotina, Mortierellomycotina, Mucoromycotina, and Zoopogomycotina. In: McLaughlin DJ, Spatafora JW, editors. Mycota VII, Part A. Systematics and Evolution. New York (NY): Springer-Verlag; 2014. p. 209–250.

[22] Schipper MAA. On Mucor circinelloides, Mucor racemosus and related species. Stud Mycol. 1976;12:1–40.

[23] Nguyen TT, Duong TT, Lee HB. Characterization of two new records of Mucoralean species isolated from gut of soldier fly larva in Korea. Mycobiology. 2016;44:310–313.

[24] Nguyen TT, Jung HY, Lee YS, et al. Phylogenetic status of two undescribed zygomycete species from Korea: Actinomucor elegans and Mucor minutus. Mycobiology. 2017;45:344–352.

[25] Souza CAFD, Lima DX, Gurgel RMS, et al. Coprophilous Mucorales (ex Zygomycota) from three areas in the semi-arid of Pernambuco, Brazil. Braz J Microbiol. 2017;48:79–86.

[26] Thompson DP, Eribo BE. Extracellular enzyme production by Rhizopus and Mucor species on solid media. Can J Microbiol. 1984;30:126–128.

[27] Alves MH, Campos-Takaki GM, Porto ALF, et al. Screening of Mucor spp. for the production of amylase, lipase, polygalacturonase and protease. Braz J Microbiol. 2002;33:325–330.

[28] de Souza PM, Bittencourt MLA, Caprraca CC, et al. A biotechnology perspective of fungal proteases. Braz J Microbiol. 2015;46:337–346.

[29] Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clin Microbiol Rev. 2000;13:236–301.

[30] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego, CA, USA: Academic Press; 1990. p. 315–322.

[31] Lee HB, Molecular phylogenetic status of Korean strain of Podosphaera xanthii, a causal pathogen of powdery mildew on Japanese thistle (Cirsium japonicum) in Korea. J Microbiol. 2012;50:1075–1080.

[32] Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–98.

[33] Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–2729.

[34] Lee YS, Jung HY, Lee HB, et al. National list of species of Korea Ascomycota, Glomeromycota, Zygomycota, Myxomycota, Oomycota.Korea: National Institute of Biological Resources Ministry of Environment; 2015.

[35] Nguyen TT, Choi YJ, Lee HB. Isolation and characterization of three unrecorded Zygomyceetes fungi in Korea: Cunninghamamella bertholletiae, Cunninghamamella echinulata, and Cunninghamamella elegans. Mycobiology. 2017;45:318–326.

[36] Schwarz P, Bretagne S, Gantier JC, et al. Molecular identification of Zygomycetes from culture and experimentally infected tissues. J Clin Microbiol. 2006;44:340–349.

[37] White MM, James TY, O’Donnell K, et al. Phylotyping of the Zygomycota based on nuclear ribosomal sequence data. Mycologia. 2006;98:872–884.

[38] Li GJ, Hyde HD, Zhao RL, et al. Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers. 2016;78:472–593.
[39] de Azevedo Santiago ALCM, de Souza-Motta CM. Isolation of Mucorales from processed maize (Zea mays L.) and screening for protease activity. Braz J Microbiol. 2008;39:698–700.

[40] Heidary M, Habibi Z. Microbial transformation of androst-4-ene-3,17-dione by three fungal species Absidia griseolla var. igachii, Circinella muscae and Trichoderma virens. J Mol Catalysis B Enzymatic. 2016;126:32–36.

[41] Álvarez E, Cano J, Stchigel AM, et al. Two new species of Mucor from clinical samples. Med Mycol. 2011;49:62–72.

[42] Simões K, Dietrich SMC, Hahn MG, et al. Purification and characterization of a phytoalexin elicitor from spores of the saprobe Mucor ramosissimus. Rev Bras Bot. 2005;28:735–744.

[43] Marques MR, Buckeridge MS, Braga MR, et al. Characterization of an extracellular endopolygalacturonase from the saprobe Mucor ramosissimus Samuseveritysch and its action as trigger of defensive response in tropical plants. Mycopathologia. 2006;162:337–346.

[44] Ko HS, Taguchi H, Takizawa K, et al. The enzymatic approach of zygomycosis – causing Mucorales. Kor J Med Mycol. 2007;12:9–17.

[45] Satari B, Karimi K. Mucoralean fungi for sustainable production of bioethanol and biologically active molecules. Appl Microbiol Biotechnol. 2018;102:1097–1117.

[46] Schwarz P, Lortholary O, Dromer F, et al. Carbon assimilation profiles as a tool for identification of zygomycetes. J Clin Microbiol. 2007;45:1433–1439.