Aspergillus cumulatus sp. nov., from Rice Straw and Air for Meju Fermentation

Dae-Ho Kim¹², Seon-Hwa Kim¹, Soon-Wo Kwon¹, Jong-Kyu Lee², and Seung-Beom Hong*¹

¹Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-853, Republic of Korea
²Tree Pathology and Mycology Laboratory, Division of Forest Environment Protection, Kangwon National University, Chuncheon 200-701, Republic of Korea

Aspergillus section Aspergillus (teleomorph Eurotium) contains economically important fungi that are widely distributed in nature and the human environments. Species belonging to this section are known for their ability to grow on substrates with low water activity, and deteriorate stored grains, cereals, and food products preserved by drying or high concentrations of salt or sugar [3]. Some species of this section are used as a starter culture in fermented food manufacturing [1, 3].

Species belonging to Aspergillus section Aspergillus were frequently isolated during studies on the mycobiota of meju, a brick of dried fermented soybeans used as starting material for soy sauce and soybean paste [1, 4, 5]. Three strains belonging to Aspergillus section Aspergillus from meju production environments could not be assigned to any known species. Therefore, we describe these strains as a new species based on morphological and molecular characteristics.

One strain was isolated from rice straw used in meju fermentation in Anseong, Korea (KACC 47316); the other two strains were from air of a meju fermentation room in Icheon (KACC 47513) and Anseong (KACC 47514) in Korea. Their morphological characters were examined according to the method of Hong et al. [2] and Hubka et al. [3]. DNA sequences of partial β-tubulin (BenA), calmodulin (Cmd), rDNA-ITS, and RNA polymerase II (Rpb2) genes were determined according to the method of Hubka et al. [3] and were compared with those of Aspergillus section Aspergillus species reported in Peterson [6], Hong et al. [2], and Hubka et al. [3]. The determined sequences have been deposited to GenBank as KF928294–KF928305.

The strains grew over a wide range of water activities. After 7 days of incubation at 25°C, growth was observed on Malt Extract agar (MEA) (a_w 0.99, 9–14 mm; Fig. 1A), Malt Extract 20% Sucrose agar (ME20S) (a_w 0.96, 43–59 mm; Fig. 1B), ME40S (a_w 0.94, 75 mm), ME50S (a_w 0.93, 53–73 mm), ME60S (a_w 0.92, >75 mm; Fig. 1C), and ME80S (a_w 0.82, 1–5 mm). At 37°C, growth was detected on ME50S [(0)8–14(40) mm], ME60S [(0)9–13 (mm)], ME70S [(9)13–27 mm], and ME80S [(0)5–6 mm] after 7 days of incubation, but not on MEA, ME20S, and DG18 (a_w 0.93).

Microscopic analysis of the strains showed the presence of yellow ascomata containing small lenticular ascospores (5.1–5.7 µm) with a wide furrow, low equatorial crests, and tuberculate convex surface. The species is phylogenetically distinct from the other reported Aspergillus section Aspergillus species based on multilocus sequence typing using rDNA-ITS, β-tubulin, calmodulin, and RNA polymerase II genes.

Keywords: Aspergillus cumulatus, new species, rice straw, Eurotium

A new species named Aspergillus cumulatus sp. nov. is described in Aspergillus section Aspergillus (Eurotium state). The type strain (KACC 47316) of this species was isolated from rice straw used in meju fermentations in Korea, and other strains were isolated from the air in a meju fermentation room. The species is characterized by growth at a wide range of water activities and the formation of aerial hyphae on malt extract 60% sucrose agar (ME60S) that resemble a cumulus cloud. Furthermore, A. cumulatus produces yellow ascomata containing small lenticular ascospores (5.1–5.7 µm) with a wide furrow, low equatorial crests, and tuberculate convex surface. The species is phylogenetically distinct from the other reported Aspergillus section Aspergillus species based on multilocus sequence typing using rDNA-ITS, β-tubulin, calmodulin, and RNA polymerase II genes.

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of small ascospores (5.1–5.7 µm) with a wide furrow, low equatorial crests, and tuberculate convex surface (Fig. 1K). Phylogenetic analysis based on combined rDNA-ITS, BenA, Cmd, and Rpb2 sequences showed that the strains form a unique clade in the *A. ruber* clade [3] (Fig. 2). The nearest species is *A. tonophilus* with 95.7% similarity. These isolates have ascospores with low crests, whereas *A. tonophilus* has no crests. Furthermore, *A. tonophilus* does not grow on MEA at 25°C, whereas these strains do.

The ascospores of the new species are similar to those of *A. proliferans* on the size, crest, furrow, and texture. However, they differ on colony size and morphology. *Aspergillus cumulatus* has grey green and yellow colonies on Czapeck Yeast Extract 20% Sucrose agar (CY20S) at 25°C, whereas *A. proliferans* has orange colonies. Furthermore, *A. proliferans* grows more slowly (15–22 mm at 7 days) than *A. cumulatus* (34–53 mm in 7 days). The two species are clearly separated by combined rDNA-ITS, BenA, Cmd, and Rpb2 sequences (94.5% similarity).

Our data show that these strains represent a new species of the *Aspergillus* section *Aspergillus* (teleomorph *Eurotium*) and will be named *Aspergillus cumulatus* sp. nov.

**Taxonomy**

*Aspergillus cumulatus* D.H. Kim & S.B. Hong, sp. nov. Fig. 1. In subgenus *Aspergillus*, section *Aspergillus* Mycobank MB807118 Etymology: L. part. cumulatus “heaped” or “piled” to describe the aerial hyphae that resemble cumulus clouds.

Colonies on ME20S grow rapidly, 43–59 mm in 7 days at