

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

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A new species named *Aspergillus cumulatus* sp. nov. is described in *Aspergillus* section *Aspergillus* (*Eurotium* state). The type strain (KACC 47316<sup>T</sup>) 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.

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

*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].

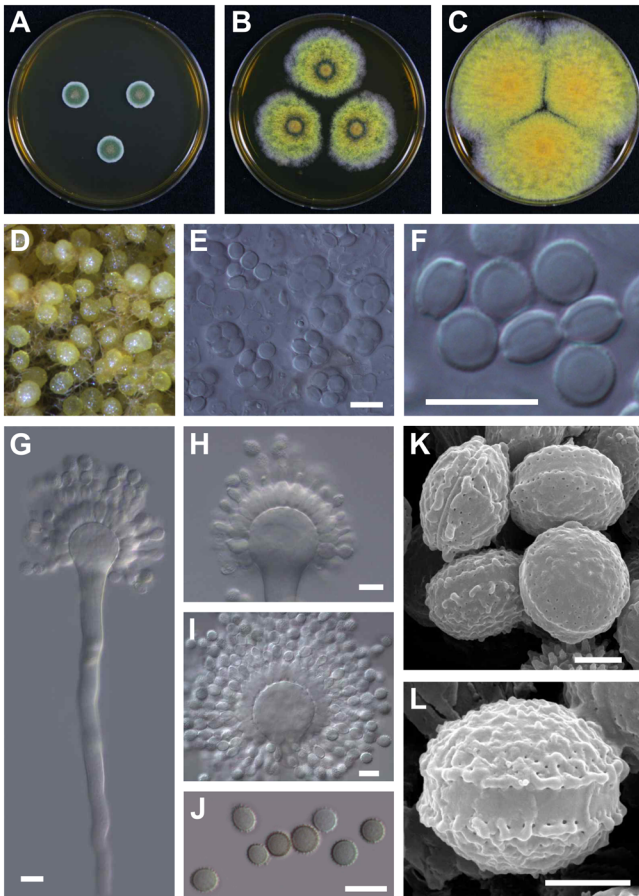
Species belonging to *Aspergillus* section *Aspergillus* were frequently isolated during studies on the mycobiota of *meju*, a brick of dried fermented soybeans used as starting material for soy sauce and soybean paste [1, 4, 5]. Three strains belonging to *Aspergillus* section *Aspergillus* from *meju* 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* sect. *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

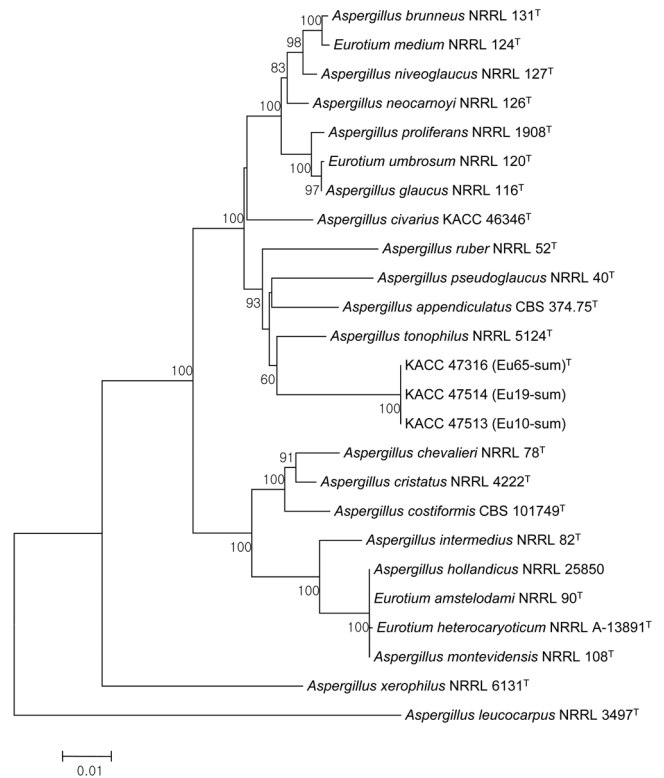


**Fig. 1.** *Aspergillus cumulatus* sp. nov., KACC 47316.

(A–C) Colonies on MEA, ME20S, and ME60S at 25°C after 7 days of incubation, (D) cleistothecia by stereomicroscope, (E) asci, (F) ascospores, (G–I) conidiophores and conidial head. (J) conidia. (K, L) ascospores by SEM. Bars: E–J = 10 µm; K, L = 2 µm.

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



**Fig. 2.** Phylogenetic position of *Aspergillus cumulatus* sp. nov. based on the combined data from rDNA-ITS,  $\beta$ -tubulin, calmodulin, and RNA polymerase II loci.

Combined data of the new species was compared with those of other species in *Aspergillus* section *Aspergillus* from Peterson [6], Hong *et al.* [2], and Hubka *et al.* [3]. The combined data were analyzed using Tamura-Nei parameter distance calculation model, and then used to construct the Neighbor-Joining (NJ) tree with MEGA ver. 5.2.2 [7]. Bootstrap analysis was performed with 1,000 replications. The “T” after the collection number indicates the type strain of the species.

*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

25°C. Yellow due to yellow cleistothecia, greyish green due to conidia and brownish orange on central part of colony due to brownish orange color hyphae. Growth on MEA restricted, 9–14 mm, centrally raised, yellow and greyish green. Colonies on DG18 spreading broadly, attaining a diameter of 49–68 mm in 7 days at 25°C, greyish green, yellow to brownish orange. Aerial hyphae and conidial heads developed. Cleistothecia are produced on whole parts except margin. Colonies on ME60S are similar with those of DG18, but produce abundant aerial hyphae that suggest cumulus clouds. No growth at 37°C on ME20S and DG18.

Homothallic. Cleistothecia, abundantly produced in 7 days, yellow, globose to subglobose, 90–175 µm in diam. Asci, 8-spored, globose to subglobose, 11–14 µm diam., evanescent at maturity. Lenticular ascospores, 5.1–5.7 µm with wide furrow, low equatorial crests, and rough convex surface on near crests (tuberculate). Conidial heads radiate. Stipe arising from aerial hyphae, smooth, 5–7 µm wide, length up to 100 µm. Vesicle globose, 10–20 µm diam. Aspergilli uniseriate, phialides ampuliform, covering entire area of vesicle. Conidia globose to ellipsoidal, often with hilum, often doliiform when young, 4.6–6.5 µm, rough.

Type strain: KACC 47316, isolated from rice straw in Anseong, Korea. The culture is permanently preserved in a metabolically inactive state (lyophilization and liquid nitrogen storage) in Korean Agricultural Culture Collection in Suwon, Korea. Molecular markers for the type strain are KF928297 for the BenA, KF928300 for the Cmd, KF928303 for the rDNA-ITS, and KF928294 for the Rpb2 genes.

Additional isolates studied: KACC 47513 and KACC 47514 (air of *meju* fermentation room; Icheon and Anseong in Korea, respectively).

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