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NOTE

Aspergillus cibarius sp. nov., from Traditional Meju in Korea

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***Aspergillus cibarius* sp. nov. isolated from meju, a brick of dried fermented soybeans in Korea, is described. The species was also found from black bean, bread and salami in the Netherlands. It is characterized by abundant yellow to reddish brown ascomata and small lenticular ascospores (4.5–5.5 µm) with a wide furrow, low equatorial crests and tuberculate or reticulate convex surface. The species was resolved as phylogenetically distinct from the other reported *Aspergillus* species with an *Eurotium* teleomorph based on multilocus sequence typing using partial fragments of the β-tubulin, calmodulin, ITS and RNA polymerase II genes.**

Keywords: *Aspergillus cibarius* (Eurotium state), new species, meju, black bean, salami

Eurotium species were isolated with a high frequency during the examination of the mycobiota of meju, a brick of dried fermented soybeans which is used as starting material for soy sauce and soybean paste (Hong *et al.*, 2011). Among those isolates, three strains could not be assigned to any known species. Using a combination of phenotypic and molecular data, we describe these isolates as a new species.

The three strains of the *Eurotium* species were isolated from meju in Icheon, Yongin, and Hoingseong in Korea (KACC no. 46346, 46764-5). Their morphological characters were examined by the methods described in Hong *et al.* (2011). In order to determine the phylogenetic position of the isolates, partial fragments of the β-tubulin, calmodulin, ITS and RNA polymerase II genes were sequenced by the methods of Peterson (2008) (GenBank Accession no. JQ918177–88) and combined. The combined sequences of three strains were compared with those of *Eurotium* species in Peterson (2008). Additionally, three strains (KACC 49766–8) of the species originated from black bean, bread and salami in the Netherlands were included in the study. The morphology and β-tubulin sequences of the Dutch strains were examined by the methods of Hong *et al.* (2011).

The species has small ascospores (4.5–5.5 µm) with a wide furrow, low but clear equatorial crests and convex surface which is rough near crests (tuberculate or reticulate) but finely rough or smooth near crown (Fig. 1I). These characteristics distinguish the species from currently recognized species of *Eurotium* (Raper and Fennell, 1965; Kozakiewicz, 1989; Kong and Qi, 1995a, 1995b; Abliz *et al.*, 2001). Based on phylogenetic tree derived from combined β-tubulin, calmodulin, ITS and RNA polymerase II data, the species was

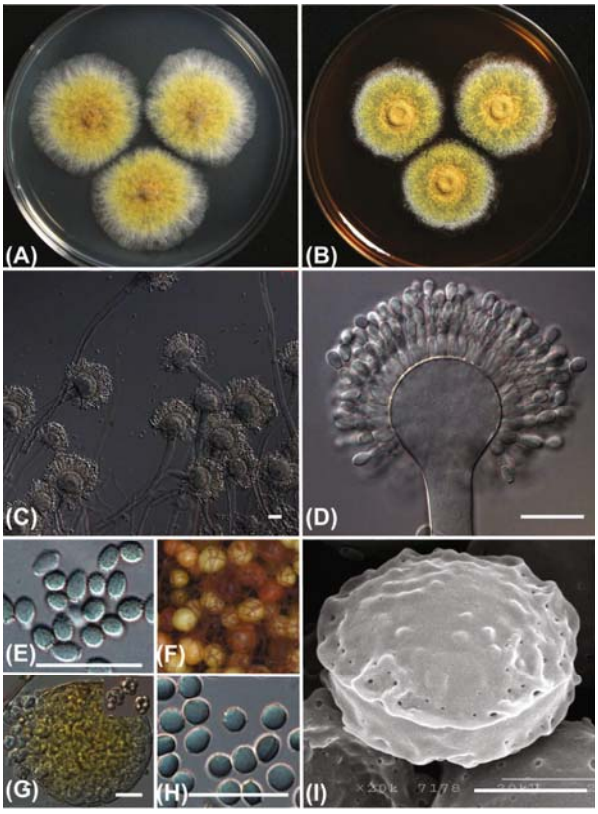

Fig. 1. ***Aspergillus cibarius* sp. nov, KACC 46346.** (A & B) Colonies on DG18 & ME20S, (C & D) Conidial head(s), (E) Conidia, (F) Cleistothecia, (G) Cleistothecium and asci, (H) Ascospores, (I) Ascospore by SEM. Size marker, 20 µm (I=2 µm).

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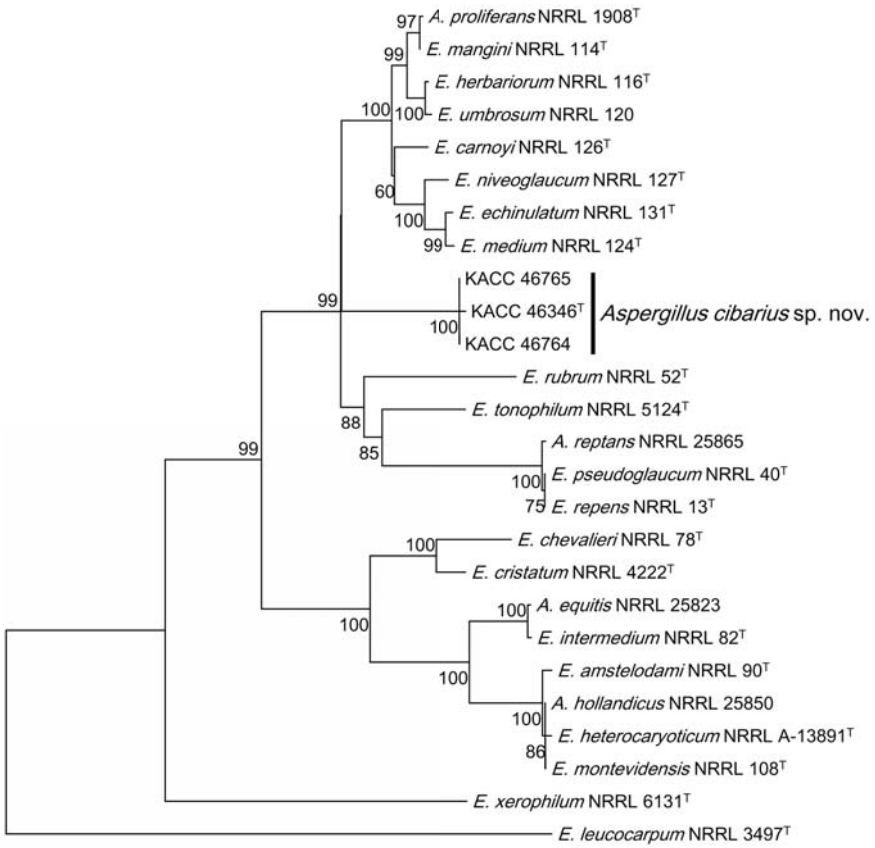

Fig. 1. Phylogenetic position of *Aspergillus cibarius* sp. nov. based on combined β-tubulin, calmodulin, ITS and RNA polymerase II data. In order to determine the position of the new species, DNA sequences of four genes in Peterson (2008) were obtained from GenBank and compared. Combined DNA data were analyzed using Tamura-Nei parameter distance calculation model, which was then used to construct the Neighbor-Joining (NJ) tree with MEGA version 5 (Tamura *et al.*, 2012). Bootstrap analysis was performed with 1,000 replications. The 'T' after the collection number indicates the type strain of the species.

clearly separated from the other *Eurotium* species examined in the study of Peterson (2008) (Fig. 2). The new species showed dissimilarity of more than 3% with the other *Eurotium* species, which is much larger than dissimilarities between *E. herbariorum* and *E. carnoyi* (1.4%) and between *E. chevalieri* and *E. cristatum* (1.7%). These morphological and molecular characteristics showed that the species represents a new species of the genus.

In July 2011, the International Code of Botanical Nomenclature (ICBN) was replaced by the International Code of Nomenclature for Algae, Fungi and Plants (ICN) (Norvell, 2011). In the new code, the dual nomenclature system was abandoned and replaced for single name nomenclature. In order to implement the new rules of the ICN, the International Commission on *Penicillium* and *Aspergillus* (ICPA) determined that the genus name *Aspergillus* will be used for all *Aspergillus* species, including those with teleomorphic state, such as *Neosartorya, Emericella*, *Eurotium*. Therefore, we decided to use the genus name *Aspergillus* for the new species in this study. However, using the old nomenclature rules, this species would have been included in the genus *Eurotium*.

Taxonomy

Aspergillus cibarius S.B. Hong & R.A. Samson, sp. nov. Fig. 1.
In: subgenus *Aspergillus,* section *Aspergillus*
Mycobank MB800861

Etymology: ci.ba'ri.us. L. masc. adj. cibarius, pertaining to food. Referring to the origin of the type strain.

Colonies on ME20S grow rapidly, 47–53 mm in 7 days at 25°C. Yellow due to yellow cleistothecia, green due to conidia and orange on central part of colony due to orange color hyphae. Growth on MEA restricted, less than 17 mm, centrally raised, yellow to orange with abundant cleistothecia. Colonies on DG18 spreading broadly, attaining a diameter of 40–48 mm in 7 days at 25°C, green, yellow to orange. Aerial hyphae and conidial heads developed. Cleistothecia produced on whole part except margin. No growth on 37°C on DG18.

Homothallic. Cleistothecia abundantly produced in 7 days, yellow, some becoming reddish brown in time, globose to subglobose, 90–175 μm in diam. Asci 8-spored, globose to subglobose, 9–12 μm diam, evanescent at maturity. Lenticular ascospores 4.5–5.5 μm with wide furrow and low but clear equatorial crests, convex surface is rough near crests (tuberculate or reticulate) but finely rough or smooth near crown. Conidial heads radiate. Stipe arising from aerial hyphae, smooth, 8–10 μm wide, length up 700 μm. Vesicle globose, 30–50 μm diam. Aspergilli uniseriate, phialides ampuliform, covering entire area of vesicle. Conidia broadly ellipsoidal to ellipsoidal, often with hilum, often doliiform when young, 5.5–6.5 μm, rough.

Type strain: KACC 46346, isolated from meju in Icheon, Korea. The culture is permanently preserved in a metabolically inactive state (lyophilization and liquid nitrogen storage) in Korean Agricultural Culture Collection in Suwon,

Korea.

Additional isolates studied: KACC 46764 (meju; Yongin, Korea), KACC 46765 (meju; Hoingseong, Korea), KACC 49766 (black bean; the Netherlands), KACC 49767 (bread; the Netherlands), KACC 49766 (salami; the Netherlands).

Note: This species was clearly separated from the other *Eurotium* species based on combined DNA sequence data of β-tubulin, calmodulin, ITS and RNA polymerase II genes. The nearest species was *E. herbariorum* (96.8% similarity) and it has 5.5–7.0 μm ascospores without equatorial crests. However, *A. cibarius* has 4.5–5.5 μm ascospores with clear crests. This species are similar with those of *E. amstelodami* in the size, crest, furrow and texture of the ascospores. However, on colony colors, *A. cibarius* is green, yellow and orange, while *E. amstelodami* is only green and yellow, because of not having orange colored hyphae. Furthermore, in contrast with *E. amstelodami, A. cibarius* does not grow on DG18 at 37°C.

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References

Abliz, P., Horie, Y., Hui, Y., Nishimura, K., and Li, R. 2001. New and interesting species of *Eurotium* from Chinese soil. *Mycoscience* **42**, 289–294.

Hong, S.B., Kim, D.H., Lee, M.A., Baek, S.Y., Kwon, S.W., and Samson, R.A. 2011. Taxonomy of *Eurotium* species isolated from meju. *J. Microbiol.* **49**, 669–674.

Kong, H. and Qi, Z. 1995a. Two new species of *Eurotium* Link. *Acta Mycol. Sinica* **14**, 10–16.

Kong, H. and Qi, Z. 1995b. Two new species of *Eurotium* isolated from Xizang (Tibet), China. *Acta Mycol. Sinica* **14**, 86–91.

Kozakiewicz, Z. 1989. *Aspergillus* species on stored products. *CMI Mycol. Pap.* **161**, 1–188.

Norvell, L.L. 2011. Fungal nomenclature. 1. Melbourne approves a new Code. *Mycotaxon* **116**, 481–490.

Peterson, S.W. 2008. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. *Mycologia* **100**, 205–226.

Raper, K.B. and Fennell, D.I. 1965. *Aspergillus glaucus* group. pp. 147–196. *In* The genus *Aspergillus*. The Williams & Wilkins Company. Baltimore, MD. USA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2012. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* (submitted).