Mycobiology 30(4): 240-243 (2002)

First Report of Bulb Canker of Garlic Caused by *Embellisia allii* in Korea

Hyang Burm Lee*, Chang-Jin Kim and Seung Hun Yu[1]

Korea Research Institute of Bioscience & Biotechnology, Yusong, Daejon 305-600, Korea
[1]Division of Applied Biology, Chemistry & Food Science, Chungnam National University, Daejon 305-764, Korea
(Received August 13, 2002)

Embellisia allii **causing bulb canker of white garlic (*Allium sativum*) has been observed on harvested or stored garlic bulbs. Identification of the fungus was determined based on the symptoms and morphological characteristics on *in vitro* potato dextrose agar (PDA) and malt extract agar (MEA). Colonies were effuse, blackish brown to black, velvety or powdery. Conidia were solitary, almost straight, most commonly ellipsoidal (or subcylindrical) to ovoid, mid to dark brown, smooth, with very dark 1 to 6 transverse (commonly 3~4) and occasionally 1 or 2 oblique or longitudinal septa, and 30.4×13.4 μm (av.). Conidiophores were simple or branched, straight or flexuous, up to 80 μm long and 5.1~10.2 μm thick. Chlamydospores were developed through enlargement and repeated cellular division of several adjacent hyphal cells to form a complex of thick-walled cells. The conidial measurements of garlic isolates closely matched the previous description of *E. allii*.**

KEYWORDS: Bulb canker, *Embellisia allii*, Garlic

Garlic (*Allium sativum*) which is a bulbous plant is used mainly as a seasoning for foods, and increasingly as a herbal medicine and pesticide in organic farming. In the course of our studies on post-harvest deterioration of garlic and screening of diverse fungal species from various isolation sources, a fungal species causing a bulb canker was observed not only on bulb cloves wrapped in polyfilm at the markets, but also on newly harvested or cultivated garlic bulbs in Korea. The wrapped cloves were collected at markets located in Daejeon and Suwon. The newly harvested or cultivated garlic bulbs were collected from patch field areas of Cheongyang, Seosan and Euiseong for the past 6 years. Lesions were up to 20 mm and generally occurred on the fleshy cloves and scales of garlic bulbs, and especially more frequently on sunken parts of bulb cloves kept under moist conditions. The cloves were covered with black conidia and often became velvety brown with the formation of dematiaceous aerial mycelia and conidia, and then depressed following collapse of tissues. Infection was done to localized or entire areas of cloves.

Single spores were easily isolated from diseased garlic bulb samples with canker using sterilized needle, and incubated on artificial media potato dextrose agar (PDA) and malt extract agar (MEA). The incidence of the disease on garlic samples varied with storage condition (cold and room temperature storage), areas collected, years harvested (data not shown). The causal fungus closely matched *Embellisia allii* (Campanile) E.G. Simmons based on descriptions of previous workers (Campanile, 1924a, b; David, 1996; Ellis, 1976; Moore, 1942; and Simmons, 1971). *E. allii* has been known as a causal agent of garlic bulb canker, especially on white garlic varieties in Europe and America (Corlett, 1996). David (1991) reported that leaves and flower stems of garlic could be also attacked by *E. allii*. Taniguchi *et al.* (1994) described common features of bulb canker occurring on the surface of the enveloping scale of the bulbs at markets in Japan, mentioning that the main symptom was characterized by scale surfaces of garlic bulb scattered with black powder. However, discoloration of fleshy inner part of bulb cloves was seldom described previously. In contrast, according to our study, the canker lesions on garlic samples were observed not only on the surface enveloping scale, but also on inner parts or surface of garlic cloves. To our knowledge, this is the first report of bulb canker on garlic cloves in Korea. The incidence of garlic bulb canker has been surveyed since the disease was first found in Korea in 1995. Although bulb canker on garlic has been considered to be a minor disease, it might be a severe matter at markets because the blackish symptoms on bulb cloves can considerably reduce commercial value.

So far, 18 species of *Embellisia* has been known, however only one species of *E. allii* has been reported from garlic in Korea (Lee *et al.*, 1999). As previously described by Simmons, *Embellisia* resembles *Alternaria*, *Bipolaris*, *Curvularia*, *Drechslera* and *Ulocladium*, but differs from them in the shape of conidia with thick and dark transverse septa. *E. allii* also differs from *E. chlamydospora* and *E. hyacinthi*. *E. chlamydospora* has conidia with apical cell which is not triangular, whereas *E. hyacinthi* has conidia with apical cell which is often triangular. *E. hyacinthi* which was first isolated from bulb scales of *Freesia*, *Hyacinthus* and *Scilla* was known to cause small

*Corresponding author <E-mail: hblee@kribb.re.kr>

necrotic spots with reddish brown margins (Ellis, 1976). The original description and illustration of *E. allii* were based on isolates on bulb scales of white garlic (Corlett, 1996; Ellis, 1976; Taniguchi, 1994). In contrast, *E. chlamydodpora* (=*Pseudostemphylium chlamydospora*) was based on isolates from underground parts of winter wheat in eastern Washington, and from wheat crowns in Northwest Territories, Canada (Hoes *et al.*, 1965; Simmons, 1971). De Hoog *et al.* (1985) reported a new species of *Embellisia*, *E. annulata* from the North Sea. Recently, a new *Embellisia* sp. identified based on inter transcribed spacer (ITS) region sequence data has been reported from the Antarctic moss *Bryum argenteum* (Bradner *et al.*, 2000). So far, it has been suggested that morphology and identification of *Embellisia* is controversial. Therefore, further studies on taxa of whole *Embellisia* species based on morphology and molecular analyses are needed.

Figure 1 and Table 1 show morphological features of *E. allii* isolated from garlic bulbs in Korea. Colonies were effuse, blackish brown to black, velvety or powdery. Conidia were solitary, almost straight, most commonly ellipsoidal (or subcylindrical) to ovoid, mid to dark brown, smooth, with very dark 1 to 6 (commonly 3~4) transverse septa, occasionally 1 or 2 oblique or longitudinal septa, and 30.4×13.4 μm (av.) on PDA. Secondary conidiophores which bear several conidia were often observed on lesions. Conidiophores were simple (or rarely branched), straight or flexuous, up to 80 μm long, 5.1~10.2 μm thick. Chlamydospores were developed through enlargement and repeated cellular division of several adjacent hyphal cells to form a complex of thick-walled cells.

Figure 2 shows mycelial growth of *E. allii* on PDA and MEA at different temperatures. *E. allii* grew fastest at 25°C. In contrast, the sporulation of *E. allii* was generally

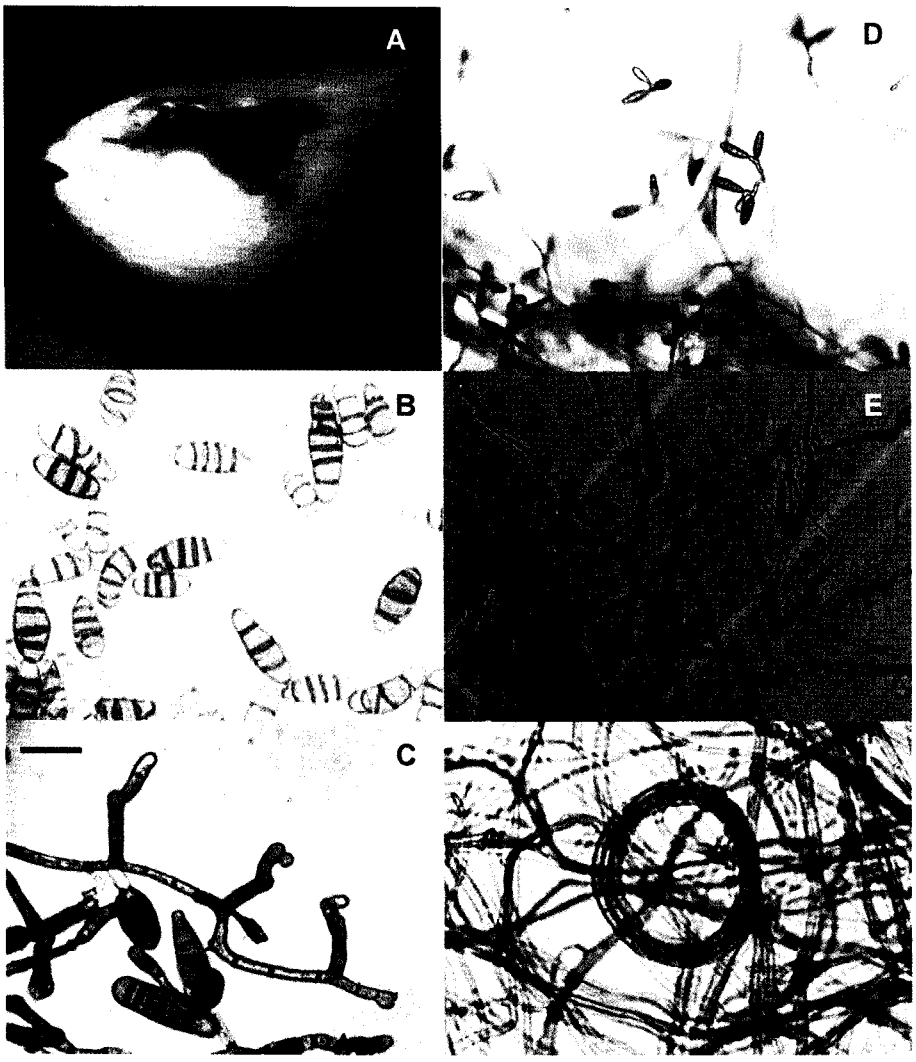

Fig. 1. A bulb canker fungus, *Embellisia allii*, isolated from garlic cloves. A: blackened canker lesion formed on a clove of garlic bulb; B and C: conidia and conidiophores from the lesion (×400); D: conidia on conidiophores in culture (×40); E: early multicellular chlamydospores on MEA at 30°C (×100); F: dematiaceous mycelia and hyphal coils on PDA (×400). Scale bar = 20 μm.

Table 1. Morphological characteristics of *Embellisa allii* isolated from garlic bulb

Characteristics	M. B. Ellis	M. Corlett	Present isolate
Colony	dark brown to black, velvety or powdery	blackish brown to black powdery	dark brown to black velvety or powdery
Conidiophores	simple or branched, up to 100 μm long and 5~10 μm thick	simple or branched, mostly 30~45 μm long, up to 100 μm long or more long, solitary or in groups of two or more	simple or branched, mostly 30~50 μm long, up to 80 μm long, solitary or in groups of two or more
Conidia	ellipsoidal or subcylindrical, 25~45× 10~15 μm, very dark 3~6 (-10) transverse and 1 or 2 longitudinal septa	ellipsoidal or somewhat cylindrical, 24~36× 9~12 μm, thick walled 4 to 8 transverse and 0 to 2 longitudinal septa, occasionally V-shaped conidia found	ellipsoidal or subcylindrical, av. 30×13 μm, dark and thick walled 1 to 6 (commonly 3~4) transverse and 1 or 2 oblique or longitudinal septa
Secondary conidiophores	short secondary conidiophores which bear conidia	secondary sporulation present in culture	secondary conidiophores which bear conidia
Chlamydospores	present on host plant, not so frequently in culture	present in culture	frequently present in culture

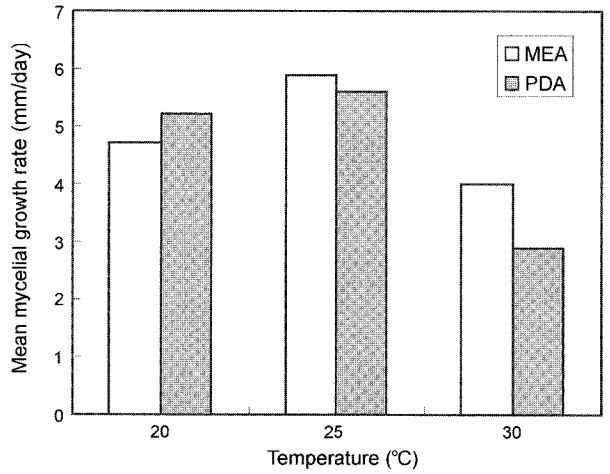

Fig. 2. Mycelial growth of *Embellisia allii* on MEA (0.995 a_w) and PDA (basic) at different temperatures.

good both on lesions of garlic bulbs and in culture, especially better at some lowered temperature (20°C) than > 25°C, but hardly observed at > 30°C regardless of media tested (data not shown). To our knowledge, bulb canker of garlic is a newly reported post-harvest disease in Korea, and we propose the Korean disease name "manul buran byoung" by *Embellisia allii*.

Importation of garlics from China and abroad has been rapidly increased in recent years. To our knowledge, it might be possible for the imported garlics to carry a range of spoilage fungi including *Embellisia*. *E. allii* was often detected along with *Fusarium* and *Penicillium* which were frequent dominants on garlic bulbs. Pathogenicity test showed that the isolate Lee01 of *E. allii* was weakly pathogenic to slightly wounded garlics when spores were inoculated on the garlic bulb cloves, as Taniguchi *et al.* (1994) previously mentioned. Further studies on the seasonal occurrence and ecology of the fungus are needed.

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