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Morphology of Hyphal Anastomosis in Entomopathogenic Fungi, *Beauveria bassiana* and *Beauveria brongniartii*¹

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Parasexual cycle and heterokaryosis have been demonstrated in entomopathogenic imperfect fungi, *Beauveria bassiana* (YURCHENKO et al., 1974), *Metarhizium anisopliae* (TINLINE and NOVIELLO, 1971; AL-AIDROOS, 1980), *Paecilomyces fumosoroseus* (RIBA and RAVELOJOANA, 1984), and *Verticillium lecanii*

(JACKSON and HEALE, 1987). The ability to form a heterokaryon in *B. bassiana* depends on fungus strains (YURCHENKO et al., 1974). The hyphal anastomosis which is necessary to analyze the mechanism of heterokaryosis has been demonstrated in *Metarhizium anisopliae* (TINLINE, 1971) and *Cephalosporium coccorum* (= *V. lecanii*) (SINGH, 1972). In this report, we describe the hyphal anastomosis in *B. bassiana* and *B. brongniartii*.

Monoconidial *B. bassiana* strain F-8 and *B. brongniartii* strain No. 22 were used. Conidia (2×10^3 conidia/ml) were suspended in 0.1 M phosphate buffer, pH 6.4 containing 0.05% Tween 80. The conidial suspension was mixed with an equal volume of melted (50°C) Sabouraud dextrose agar plus 0.5% yeast extract (glucose, 40 g; peptone,

Fig. 1. Hyphal anastomosis in *Beauveria bassiana* strain F-8. A: H-shaped anastomoses between parallel-running hyphae; B: Clamp connection structure; C: Intrahyphal anastomosis forming ring-shaped structure; D: Conidiophore developed from an anastomosed cell. Bar = 10 μ m.

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Fig. 2. Hyphal anastomosis in *Beauveria brongniartii* strain No. 22. A: Induction of projections on hyphae; B: Fusion between parallel-running hyphae and intrahyphal anastomosis forming a ring-shaped structure; C: Fusion between hypha and anastomosed cell (Y-shaped structure); D: Intrahyphal anastomosis forming ring-shaped structure. Bar = 10 μ m.

10 g; yeast extract, 5 g; agar, 20 g; distilled water, 1,000 ml), and layered on a sterilized slide glass. The conidia were incubated at 25°C in the moist chamber and observed daily under a light microscope for 7 days.

The hyphal anastomosis of each fungus species was observed at several parts of a mycelium and a hyphal net-work was formed. Usually, several hyphal anastomoses occurred on two branched

hyphae near the branched point or on two parallel-running hyphae (H-shaped anastomosis) (Figs. 1A and 2A). However, the hyphal anastomosis was not observed at hyphal tips and the edge of colony where hyphae were growing vigorously and the frequency of hyphal anastomosis was high in old hyphae. Several projections induced on hyphae grew toward another projection or anastomosed cells (Fig. 2A).

Besides interhyphal anastomosis, the following intrahyphal anastomoses were observed. i) An elongated projection fused with the original hypha similar to the clamp connection in higher basidiomycetes (Fig. 1B). However, this type of anastomosis was rarely seen. ii) Two projections which elongated from the same hypha fused to form a ring-shaped structure (Figs. 1C, 2B, and 2D). This type of anastomosis occurred more frequently in *B. brongniartii* strain No. 22 than in *B. bassiana* strain F-8. The ring structure in *B. brongniartii* strain No. 22 was larger than that in *B. bassiana* strain F-8. In *B. brongniartii* strain No. 22, the hyphal fusion occurred occasionally between a bridge cell formed by intrahyphal anastomosis and an another hypha, resulting in a Y-shaped structure (Fig. 2C). The production of conidiophore from the anastomosed cell was observed (Fig. 1D). Conidia produced from such a conidiophore will be utilized for the isolation of diploids and recombinants.

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