Successful Development of *Cordyceps bassiana* Stromata from *Beauveria bassiana*

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A specimen of *Beauveria bassiana* was collected from Yang-yang of Gangwon province, Korea in October 2006. Conidial isolates were prepared from the specimen by the dilution method and inoculated in brown rice medium for fruiting body production. After nearly two months incubation for perithecial stromata developed from single isolates as well as from their combinations. They were determined as *Cordyceps bassiana* by observing the stromatal characters and their conidial structures. This is the first report of the development of *C. bassiana* from *B. bassiana* cultures.

KEYWORDS : *Beauveria bassiana*, Conidial isolates, *Cordyceps bassiana*, Perithecial stromata

Beauveria was first shown as anamorphic state of *Cordyceps* by Shimazu *et al.* [1]. At the time of its report, perithecial stromata of *C. brongniartii* were artificially produced from scarabaeoid cadavers that was infected by *B. brongniartii*. For a long time, *C. brongniartii* was not found in the wild, until it was identified by Sasaki *et al.* [2] recently reported it from Hokkaido, Japan. Besides Japan, *C. brongniartii* has only reported in China [3], which has been indetail discussed by Sasaki *et al.* [2]. *B. brongniartii* is a global species, but its teleomorphic state is a rare occurrence in nature, This is an example that shows some *Cordyceps* species survive in nature predominantly in their anamorphic state. This is also supported by a recent discovery of another *Cordyceps* species, *C. bassiana*, from China, which was shown as the teleomorph of *B. bassiana* [4, 5]. *B. bassiana* is one of the most common mitosporic entomopathogenic fungi in the world. Both reports indicate that the teleomorphic states of *Beauveria* are also very rare in nature while and then the anamorphic states are predominant. Shimazu *et al.* [1] have successfully begun the process of discovering teleomorphs from anamorphs by incubating them under controlled environmental conditions. Recently, *C. bassiana* has been reported from Korea and has been successfully produced in culture [6, 7].

In the present study, isolates of *B. bassiana* were tested for fruiting body production in culture. Perithecial fruiting bodies produced from *B. bassiana* were found as similar to those of *C. bassiana* [5, 7]. Perithecia, ascospores, and conidial structures were also found as similar. Here,

we report the successful induction of perithecial stromata of *C. bassiana* from *B. bassiana* isolates for the first times.

Materials and Methods

Fungal isolates. A specimen of *B. bassiana* EFCC 13188, growing on adult mantis, was collected from Yang-yang of Gangwon province, Korea in October 2006 and preserved in the Entomopathogenic Fungal Culture Collection (EFCC), Kangwon National University, Korea (Fig. 1). After the collection, the conidia were transferred from the fresh specimen to 2% water agar (WA) plates in two parallel lines with the help of a sterile insect pin and incubated at 25°C under continuous light. The WA plates were regularly observed for the conidia germination and hyphal growth. After two days of incubation, fine, regular hyphal growths could be observed by the naked eye on the sides of the parallel lines. Small agar blocks containing hyphal growths were cut with the help of a Zeiss dissecting microscope Stemi SV11 (Zeiss, Oberkochen, Germany)

Fig. 1. Specimen of *Beauveria bassiana* EFCC 13188.

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and then transferred to of half strength Sabouraud's dextrose *agar* yeast extract (SDAY) agar plates (dextrose 20 g, peptone 5 g, yeast extract 5 g and agar 15 g per 1,000 mL; pH 5.6). The agar plates were incubated at 25°C under continuous light. Mycelial discs (4 mm) from the peripheral region of the two-week old agar cultures were inoculated in flasks of SDAY broths (SDAY without agar) and incubated in stationary condition under light for three days. The broths cultures were occasionally shaken manually. The culture suspension was diluted in 1.8 mL Eppendorf tubes in different grades and then plated in WA plates. After two days of incubation, single conidial colonies were isolated from the WA plates through a Zeiss dissecting microscope Stemi SV11 (Zeiss) and transferred to half-strength SDAY agar plates. The agar plates were incubated at 25°C under light. Eight single conidial isolates were selected and used in the present study. The isolates were numbered from EFCC 13188-1 to EFCC 13188-8.

Fruiting body formation. All of the eight single conidial isolates, EFCC 13188-1 to EFCC 13188-8 incubated for the induction of fruiting bodies, following the method of Shrestha *et al.* [8]. After regular observations until fifty-five days of incubations, very surprisingly only few combinations, including two single inoculations, produced perithecial stromata (Table 1, Fig. 2). Perithecial stromata produced from *B. bassiana* isolates were compared with those of *C. bassiana* [5, 7]. Similarly, conidial structures

Table 1. Fruiting body formation from single conidial isolates of *Beauveria bassiana* EFCC 13188

Isolate no.	1	2	3	4	5	6	7	8
1	–	–	–	+	+	–	–	–
2		+	–	–	–	–	–	–
3			–	–	–	–	–	+
4				+	+	+	+	–
5					–	–	–	+
6						–	–	–
7							–	–
8								–

+ indicates perithecial stromata formation, – indicates non-perithecial fruiting body formation.

Table 2. Fruiting bodies formation from *Beauveria bassiana* single conidium isolates

Isolates	<i>Cordyceps bassiana</i> EFCC 12511a × b				
	1	2	3	4	
<i>Beauveria bassiana</i> EFCC 13188	1	–	–	+	–
	2	–	–	+	–
	3	–	–	–	–
	4	–	–	–	–
	5	–	–	–	–
	6	–	–	–	+
	7	–	+	–	–
	8	–	–	–	–

+ indicates perithecial stromata formation, – indicates non-perithecial fruiting body formation.

Fig. 2. Fruiting body formation from single conidial isolates of *Beauveria bassiana* EFCC 13188. A, H3188-1 × 4; B, 13188-1 × 5; C, 13188-2; D, 13188-3 × 8; E, 13188-4; F, 13188-4 × 5; G, 13188-4 × 6; H, 13188-4 × 7 and I, 13188-5 × 8.

of *B. bassiana* EFCC 13188 were compared with those of *C. bassiana* 12511 (Fig. 4).

Out-crossing between *B. bassiana* and *C. bassiana*. All of the eight single conidial isolates of *B. bassiana* EFCC 13188 and four single ascospore isolates of *C. bassiana* EFCC 12511a × b [6] were inoculated in brown rice medium in all possible combinations of two isolates at a time to observe the nature of fruiting bodies (Table 2). The fruiting bodies were observed for perithecia development after fifty days of incubation.

Results and Discussion

Morphological comparison between *B. bassiana* and *C. bassiana*. Single conidial isolates of *B. bassiana* EFCC 13188 were similar to *C. bassiana* EFCC 12511 isolates [6] with regard to colony pigmentation and texture. Three combinations of isolates of *B. bassiana* EFCC 13188-1 × 5, 3 × 8, and 5 × 8 produced perithecial stromata (Table 1, Fig. 2). Isolate EFCC 13188-4 produced perithecial stromata when inoculated in single as well as in combinations with isolates EFCC 13188-1, 5, 6, and 7, but not with isolates EFCC 13188-2, 3, and 8 (Table 1). However, isolate EFCC 13188-2 produced perithecial stromata in single, but produced no perithecial stromata with any other isolates of *B. bassiana* (Table 1). It could not be understood if co-inoculations of two isolates suppressed fruiting body formation or not. Mating type was found to be very variable among the isolates, which is in contrast with *C. militaris* that shows more stable mating types [8]. Thus, despite the formation of perithecial stromata, *B. bassiana* isolates could not be separated into distinct mating types. This may be due to the dominance of an asexual life cycle in *B. bassiana*.

Morphological characteristics of perithecial fruiting bodies produced from *B. bassiana* EFCC 13188 isolates were compared with those of *C. bassiana*. The stromatal characteristics and size of perithecia and ascospores were

Fig. 3. Cross-section of the perithecial stromata produced from *Beauveria bassiana* EFCC 13188 isolates.

Fig. 4. Conidial structures of *Cordyceps bassiana* EFCC 12511 (left) and *Beauveria bassiana* EFCC 13188 (right). Scale Bar = 10 μ m.

within the range of *C. bassiana*, as reported by Li *et al.* [5] and Sung *et al.* [7] (Fig. 3). Conidial structures of *B. bassiana* EFCC 13188 were also similar to those of *C. bassiana* EFCC 12511 (Fig. 4). Thus, *B. bassiana* EFCC 13188 was confirmed as the anamorph of *C. bassiana*.

Out-crossing between *B. bassiana* and *C. bassiana*. Four out-crossings between *B. bassiana* and *C. bassiana*, EFCC 13188-1 × EFCC 12511a × b 3, EFCC 13188-2 × 12511a × b 3, EFCC 13188-6 × 12511a × b 4 and EFCC 13188-7 × 12511a × b 2, produced perithecial stromata (Table 2, Fig. 5). Interestingly, *B. bassiana* isolate EFCC 13188-2 produced no perithecial stromata with any other sister isolates of *B. bassiana* 13188 (Table 1), but it produced perithecial stromata with *C. bassiana* EFCC 12511a × b 3 (Table 2, Fig. 5).

Out-crossing between EFCC 13188-7 × EFCC 12511a ×

Fig. 5. Fruiting body formation from out-crossings between *Beauveria bassiana* EFCC 13188 and *Cordyceps bassiana* EFCC 12511a × b. A, EFCC 13188-1, EFCC 13188-1 × EFCC 12511a × b 3, EFCC 12511a × b 3; B, EFCC 13188-2, EFCC 13188-2 × EFCC 12511a × b 3, EFCC 12511a × b 3; C, EFCC 13188-6, EFCC 13188-6 × EFCC 12511a × b 4, EFCC 12511a × b 4, and D, EFCC 13188-7, EFCC 13188-7 × EFCC 12511a × b 2, EFCC 12511a × b 2.

b 2 showed excellent fertile fruiting body production (Fig. 5d). Mating types of both *B. bassiana* EFCC 13188 and *C. bassiana* EFCC 12511a × b isolates could not be verified from out-crossings. It is quite difficult to study also mating system of rare *Cordyceps* species, such as *C. bassiana*. From the present study, it has been shown that hyphomycetous entomopathogenic fungi can induce the production of teleomorphic states of *Cordyceps* under the suitable nutritional and environmental conditions.

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