



Morphological and molecular identification of *Beauveria bassiana* from agricultural soils

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ABSTRACT

The soil environment is an important reservoir for a wide variety of entomopathogenic fungi, which can significantly contribute to the control of insect populations, especially on agricultural pests. Comparison of entomopathogens with conventional chemical pesticides, there are advantages in using biological control agents, such as human safety and other non-target organisms; pesticide residues are minimized in food and biodiversity increased in managed ecosystems. The main objective of this study is to detect and identify the entomopathogenic fungi from agriculture soils based on morphological and molecular identification. Total of 115 soil samples were collected in spring (before sowing) from different crop fields in “Unjin” and “Nart” Research and Education Centers of the university, which are located in Bornuur sum, Tuv Province from 2016 to 2018. Isolation of entomopathogenic fungus, *Beauveria bassiana* was isolated using Dilution plate method in Peptone Dextrose Agar Yeast. Based on microscopic observation, hyphae branched and formed conidiogene cells, and single cell *B. Bassiana conidium* was round and tend to oval with hyaline color. Colonies on PDAY were white to pale yellow and sometimes red pigments in reverse. Using PCR method with primer specific for identification *B. bassiana*, molecular analysis confirmed that all six isolates has same size of band which, appeared on agarose gel.

KEYWORD: Insect, crop rotation, entomopathogenic fungi, PCR

INTRODUCTION

Over few decades, synthetic chemical pesticides have been mainly used for pest control. However, their effects on non-target organisms, residues on crops, pest resistance and concern over the environmental impact of agricultural inputs give urgency to the search for an alternative, biologically based forms of pest control [1]. Interests in microbial insecticides as biological control between 1980 and 1990, usage and safety aspects are discussed more detail in publications by Hall et al. [2] and Laird et al. [3]. As seen the advantages in using microbial control agents, the importance of entomopathogens has been highlighted as an environmentally friendly pest control method [4].

Approximately 750 species of entomopathogenic fungi are known from 85 genera found throughout the classes of fungi [4]. Most of the taxonomic groups contain entomopathogenic genera, such as *Metarhizium*, *Beauveria*, *Verticillium*, *Nomuraea*, *Entomophthora*, and *Neozygites*, and few others. One of the best-known genera of entomopathogenic fungi is *Beauveria bassiana*, which is used for biological control of insect pests.

Generally, the main difference between the most common species of entomopathogenic fungus has

been characterized based on their shape and size of colony and conidia. However, it is still problematic due to the relatively large heterogeneity of spherical *B. bassiana*. Conidial morphology and development criteria are commonly used to identify and classify genus level as *Beauveria* spp. Due to these difficulties on morphological identification a molecular technique was developed to assist complementary identification of the fungus [5].

There are few studies have been done on entomopathogenic fungus *B. bassiana*, as well as determination of biological activity for *Metarhizium anisopliae* [6]. In Mongolia, local strains of fungus *B. bassiana* Bb-G07 and Bb-G10 has been isolated from naturally infected locusts in 2011 [7].

Soil is the main source and natural habitat for entomopathogenic fungi [8]. Hence, the main goal of the present study is to detect and identify the entomopathogenic fungi *B. bassiana* in agricultural soils with different crops based on morphological and molecular identification. In this study, molecular analysis with multiplex PCR in order to confirm the result of morphological identification has been performed.

MATERIALS AND METHODS

A total of 115 soil samples were collected between 2016-2018 from different crop fields in “Unjin” and “Nart” Research and Education Centers of the university, which are located in Bornuur sum, Tuv Province, Mongolia. In each sampling site, soil samples (1.5-2kg) were collected from selected 5 points of 0-20 cm in soil depth. Sub-soil samples were mixed together to make it homogeneous. Then, the soil was sieved and stored at 4°C in the dark before use.

Isolation and morphological examination have been done at the Laboratory of Plant diseases, School of Agroecology, Mongolian University of Life Sciences. Molecular identification of PCR analysis has been done in Genetic Laboratory of Seoul University, South Korea. For the *B.bassiana*, it was isolated using dilution plate method in Potato Dextrose Agar Yeast (PDAY). At first, isolation of *B.bassiana* was identified based on their morphological characterizations. Morphological characters of the colonies include the growth pattern, color, shape, surface texture, colony elevation, and time that colony needed to cover up the petri dish. Microscopic observations of *B. bassiana* were characterized such as shape, color of hyphae and conidia as referred by Kulu et al [1].

Genomic DNA was extracted with the established CTAB method [9]. Briefly, cell walls of fungal mycelia were broken down by grinding with glass rods or in the presence of liquid nitrogen. The CTAB extraction buffer was then added, and after incubation at 65°C, purification with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitation with isopropanol were conducted. Finally, the DNA was dissolved in 50 µl of pure water.

To confirm the identity of the causal fungus, the complete ITS rDNA of the representative fungal pathogen was amplified and sequenced using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as described by White et al. [10].

PCR reaction mixture (totally 50 µl) was consisted of 1 µl from of dNTPs mixture, 1 µl of each 2 primers, 25 µl Green Taq master mix (all from AB gene), 3 µl of genomic DNA and 19 µl pure water. The My Genie™ 32 Thermal Block, Bioneer was programmed as follows: 5 min at 95°C for initial denaturation; 35 cycles of denaturation for 35 s at 94°C, annealing for 30 s at 58°C, extension for 30 s at 72°C; and a final extension for 10 min at 72°C.

RESULTS AND DISCUSSION

Morphological identification of *B. bassiana*

B. bassiana were isolated from 6 isolates among 570 samples from soils in fruit, vegetables and wheat

fields of Bornuur sum. Morphological characterization of *B. bassiana* isolates are shown on Table 1.

Table 1.

Morphological characters of *Beauveria bassiana* isolations

| Isolate code | Sample location | Colony Observation | | | | |
|------------------|------------------------------|--------------------|-------|---------------|---------|-----------------|
| | | Growth pattern | Color | Shape | Texture | Elevation |
| B.b | /N/Wheat field* | Disperse | White | Round | Smooth | Raised |
| Bu | /U/ Potato field | Disperse and dense | White | Round | Smooth | Raised |
| 1-1-3 | /N/ Fruit field | Disperse | White | Round | Smooth | Raised |
| 3-1-3 | /N/ Wheat field ⁺ | Disperse | White | Round | Smooth | Raised |
| 4-2-2 | /N/ Wheat field ⁻ | Disperse | White | Round | Smooth | Raised |
| 4-3-3 | /N/ Wheat field ⁻ | Disperse | White | Round | Smooth | Raised |
| Kulu et al. 2015 | | Disperse and dense | White | Round, Dotted | Smooth | Raised and flat |

/N/- “Nart” Research and Education Center, /U/- “Unjin” Research and Education Center,

* - no rotation, ⁺ - fallow field used to wheat and wheat rotation field/, - - fallow field used to no rotation wheat field

Morphological characterization of *B. bassiana* isolates have shown the following, as colony color was white and texture was smooth as like powder, and it is identical when colony growth more. Among six isolates, there were no significant differences on color

and texture. This result was coincided to Kulu et al. which shown that *B. bassiana* will grow on PDA medium as white mycelium and form white powder layer (Fig. 1, 2).



Figure 1. Morphological characterization of *B. bassiana* isolation.

A) Colony morphology of *B. bassiana*; B) Mycelium C) Conidia and phialides D) Conidia

Based on microscopic observation, hyphae have branched and formed conidiogene cells, and branch

long. Single cell *B. bassiana* conidium was round and tends to oval.

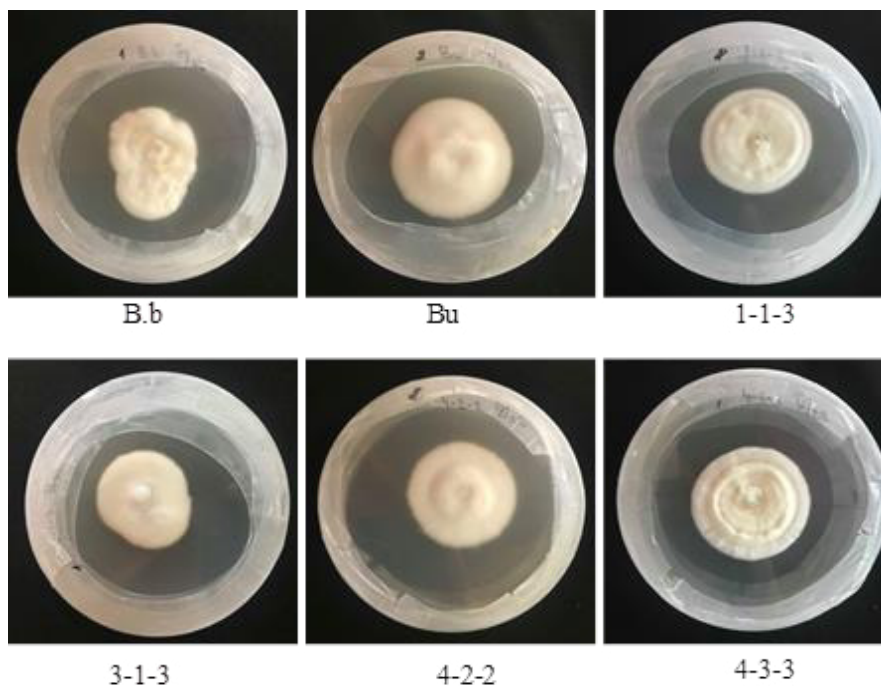


Figure 2. Pure isolations of soil samples taken from different crop fields.

Molecular identification of *B. bassiana* in PDAY medium after 21 days of culture

Molecular analysis confirmed the result of morphological identification and it indicated that entomopathogenic fungi *B. bassiana* preserved in soils from fruit and no rotation wheat fields located in *Nart*, and potato field of *Unjin*, respectively.

Six fungal isolates located at the same band and were

characterized by sequencing of the internal transcribed spacer (ITS) rDNA using ITS1 and IT4 primers (Fig 3). Isolate sequences had 100% homology to *Beauveria bassiana*. The nucleotide sequences were deposited in GenBank (KT310219, KR998521, KR998520)

<https://www.ncbi.nlm.nih.gov/nucleotide/1293835097>

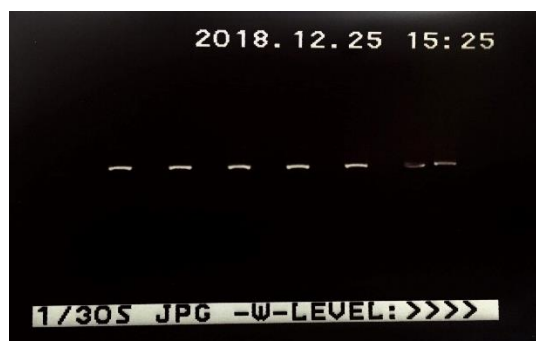


Figure 3. All isolates have same bands on agarose gel
1-B.b, 2-Bu, 3-1.1.3, 4-3.1.3, 5-4.2.2, 6-4.3.3

DISCUSSION

Based on morphological and molecular characterization of *B. bassiana* found in different crop fields, the results indicate that agricultural soils are an important reservoir of entomopathogenic fungi.

Project on biological control of insects using entomopathogenic fungi must consider the availability and acquisition the cost of virulent strains from germplasm collections [4]. In this aspect, it is important to explore local environments to detect promising, virulent fungal strains. This is the primary

stage in the development of bio-pesticide for biological management.

Several studies suggest that soil texture, acidity and organic matter content led to progressively higher percentages of entomopathogenic fungi in soils [8]. Quesada-Moraga et al. 2007, concluded that absence and less occurrence of *B. bassiana* due to the less content of organic matter [9].

Therefore, further study focused more about the insect-pathogen dynamics in soils, and ecology of entomopathogenic fungi in different soil types and in different geographical regions are necessary.

CONCLUSION

Based on morphological and molecular identification of fungi *B. bassiana* were found in agricultural soils with different crops. There are no morphological and molecular differences between *B. bassiana* isolates.

Our study indicated that agriculture soils are an important reservoir of entomopathogenic fungi and its potential for using as biological control of pests.

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