Beauveria majiangensis, a new entomopathogenic fungus from Guizhou, China

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Abstract

Beauveria majiangensis sp. nov., a fungal grub parasite, isolated from a blueberry farm in Guizhou Province, China, is herein described based on morphological and phylogenetic evidence. Beauveria majiangensis differs from other Beauveria species based on its indeterminate, denticulate rachis, cylindrical or sometimes subspherical conidiogenous cells, and ellipsoidal conidia. Phylogenetic analyses based on four loci (TEF, RPB1, Bloc, and ITS) strongly support that this strain is distinct within Beauveria.

Key words: Beauveria, grub, morphology, phylogeny, Coleoptera

Introduction

Beauveria is one of the most ubiquitous anamorphic genera of entomopathogenic fungi, and includes ecologically and economically important species (Posada & Vega 2005, Ownley et al. 2008, Roy et al. 2010); however, some Beauveria are endophytes or saprobes (Vega et al. 2008, Moonjely et al. 2016). Members of Beauveria have branched, penicillate or trichodermoid conidiophores. Dense clusters of sympodial and globose or flask-shaped short conidiogenous cells, with an apical denticulate rachis, form on conidiophores and give rise to single-celled, hyaline conidia (Chen et al. 2013, Rehner et al. 2011).

The host taxa in which pathogenic Beauveria have been found include Araneae, Blattariae, Coleoptera, Diptera, Embioptera, Heteroptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mantodea, Neuroptera, Orthoptera, Siphonaptera, and Thysanoptera (Zimmermann 2007, Chen et al. 2017). There are 11 species with hosts in the order Coleoptera: B. bassiana (Bals.-Criv.) Vuill.; B. brongniartii (Sacc.) Petch; B. amorpha (Höhn.) Minnis, S.A. Rehner & Humber; B. asiatica S.A. Rehner & Humber; B. caledonica Bissett & Widden; B. malawiensis S.A. Rehner & Aquino de Muro; B. pseudobassiana S.A. Rehner & Humber; B. sungii S.A. Rehner & Humber; B. varroae S.A. Rehner & Humber; B. lii Sheng L. Zhang & B. Huang; and B. hoplochelii I. Robêne-Soustrade & S. Nibouche.

Recently, entomopathogenic fungi were screened in Guizhou, China, and we isolated a grub-infecting Beauveria strain. Based on morphological characteristics and phylogenetic analysis, we concluded that this strain represents a new species and is described herein as B. majiangensis.

Materials and methods

Specimen collection and isolation

In December 2015, a fungus-infected grub specimen (GZU1214) was collected from a blueberry farm in Majiang, Qiandongnan Prefecture, Guizhou Province, China, by Man Liu of the Guizhou Institute of Biology. Strain GZU12141 was isolated from this infected grub specimen on improved potato dextrose agar (PDA, with 1% w/v peptone).
FIGURE 1. Phylogenetic analysis of GZU12141, GZU12142, and related Beauveria species based on combined partial TEF+RPB1+Bloc sequences. Statistical support values (≥50 %) are shown at nodes, and present bootstrap values/Bayesian posterior probabilities.
**Strain culture and identification**

Strain GZU1214 was incubated on Sabouraud’s dextrose and potato dextrose agars at 25°C for 14 d. Morphological characteristics of the fungus were examined using classical mycological techniques based on growth rate, and macroscopic and microscopic characteristics. The ex-type culture and a dried-culture holotype specimen are deposited in GZAC, Guizhou University, Guiyang, China.

**DNA extraction, PCR amplification, and nucleotide sequencing**

DNA extraction was performed according to Liang et al. (2009). The extracted DNA was stored at -20 °C. Taq enzyme and dNTP were from Shanghai Tiangen. Internal transcribed spacer (ITS), RNA polymerase II largest subunit (RPB1), B locus intergenic region (Bloc), and translation elongation factor 1 alpha (TEF), were amplified by polymerase chain reaction (PCR) according to the procedures described by White et al. (1990), Castlebury et al. (2004), Rehner et al. (2006), and van den Brink et al. (2012), respectively. PCR products were purified using the UNIQ-10 column PCR products purification kit [no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China] according to the manufacturer’s protocol and sequenced with the above PCR products at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

**Sequence alignment and phylogenetic analyses**

DNA sequences generated in this study were assembled and edited using Lasergene 6.0 (DNASTAR, Madison, WI, USA). TEF, RPB1, and Bloc sequences from 19 taxa (18 Beauveria isolates and one Isaria cicadae strain as outgroup), and ITS sequences from 20 taxa (19 Beauveria isolates and one I. cicadae strain as outgroup) were downloaded from GenBank, based on Agrawal et al. (2014), Ariyawansa et al. (2015), Chen et al. (2013), Chen et al. (2017), Imoulan et al. (2016), Robène-Soustrade et al. (2016), Rehner & Buckley (2005), Rehner et al. (2011), and Zhang et al. (2012). Multiple sequence alignments for TEF, RPB1 and Bloc were carried out using MAFFT v7.037b (Katoh et al. 2013). Sequence editing was performed with MEGA6 (Tamura et al. 2013) and the resulting output was in Fasta file format. The concatenated TEF+RPB1+Bloc sequences were assembled by SequenceMatrix1.7.8 (Vaidya 2011). Gene concordance was assessed with the ‘hompart’ command in PAUP4.0b10 (Swofford 2002).

The combined three-gene (TEF+RPB1+Bloc) and ITS dataset was phylogenetically analyzed using MrBayes 3.2 (Ronquist et al. 2012). Two runs were simultaneously executed for 10,000,000 generations, saving a tree every 500 generations. The GTR+I+G and HKY+I+G nucleotide substitution models were used for TEF+RPB1+Bloc and ITS sequences, respectively, which were the best-fit substitution models for maximum likelihood analysis. The
GTRGAMMA model was used for all partitions in accordance with RAxML manual recommendations against invariant site use. All phylogenetic reconstructions were performed on the CIPRES web portal (Miller et al. 2010). The final alignment is available from TreeBASE (ID 21673).

Results

Phylogenetic analyses

*TEF*, *RPB1*, *Bloc* and *ITS* sequencing from GZU12141 was successful (GenBank accession no.: MG052640, MG052644, MG052639, and MG052642, respectively). The alignment lengths and number of taxa sampled for *TEF*+*RPB1*+*Bloc* and *ITS* from GenBank were 2710 bp, from 19 taxa and 425 bp from 20 taxa, respectively. Strains GZU12141 (and a second isolate, GZU12142), formed a single clade in both combined data (*TEF*+*RPB1*+*Bloc*) and *ITS* analyses (Figs 1, 3).

Taxonomy

**Beauveria majiangensis** W.H. Chen, M. Liu, Z.X. Huang, G.M. Yang, Y.F. Han, J.D. Liang & Z.Q. Liang sp. nov. (Fig. 2) MycoBank No.: MB823150

Type:—CHINA. Guizhou Province: Qiandongnan Prefecture, Majiang (N 26°42'47", E 107°43'37"), on a grub (Coleoptera: Scarabaeoidea) in blueberry farm, 14 December 2015, Man Liu, holotype GZAC GZU1214, ex-type culture GZAC GZU12141.

Colony growth and appearance similar on full-strength Sabouraud’s dextrose and potato dextrose agars, 30.5 mm in diam., after 14 d at 25 °C, non-odorous, aerial mycelium white, dense, velutinous, powdery while sporulating; white to yellowish white. Reverse light aurantium in older potions. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1.6–2.2 μm wide. Conidiogenous cells solitary or occurring in lateral clusters, base cylindrical or sometimes subspherical, 3.8–12.9 (–16.2) × 1.2–1.5 (–2.3) μm, apex with an indeterminate, denticulate rachis less than 1 μm wide. Conidia 2.4–3.8 × 1.6–2.3 μm, Q = 1.6–2.6 (Lm = 2.1, Wm =1.4, Qm =1.6), ellipsoidal, hyaline, aseptate, walls smooth and thin.

Etymology:—*majiangensis* named after the place, Majiang from which the fungus was collected.

Additional specimens examined:—CHINA. Guizhou Province: Qiandongnan Prefecture, Majiang (N 26°42’47", E 107°43’37"), on a grub (Coleoptera: Scarabaeoidea) from a blueberry farm, 14 December 2015, Man Liu (GZAC GZU12142). Sequences from this strain were deposited in GenBank under accession numbers: MG052641= *TEF*, MG052645= *RPB1*, MG052638= *Bloc*, and MG052643= *ITS*.

Known distribution:—Guizhou Province, China.

Discussion

As originally described by de Hoog (1978), the main characters for *Beauveria* are basally inflated conidiogenous cells that sympodially produce conidia on divergent denticles. Based on these characteristics, strain GZU12141 clearly belongs to *Beauveria*. Six other *Beauveria* species produce an indeterminate, denticulate rachis similar to *B. majiangensis*: *B. caledonia*, *B. gryllotalpidicola*, *B. lii*, *B. loeiensis*, *B. medogensis*, and *B. rudraprayagi*. A comparative summary of the main characters of *B. majiangensis* and these other six species is provided (Table 1). *Beauveria majiangensis* is distinguished from *B. gryllotalpidicola*, *B. lii*, *B. loeiensis*, *B. medogensis*, and *B. rudraprayagi* based on its ellipsoidal conidia and their size. *B. majiangensis* is easily distinguished from *B. caledonia*, the latter possessing ellipsoidal to more or less cylindrical to conoidal conidiogenous cells. Thus, morphological characters confirm that *B. majiangensis* is a new *Beauveria* species.
FIGURE 3. Phylogenetic analysis of GZU12141, GZU12142, and related Beauveria species based on ITS sequences. Statistical support values (≥50 %) are shown at nodes, and represent maximum likelihood bootstrap values/Bayesian posterior probabilities.
TABLE 1. Comparison of morphological characters between Beauveria majiangensis and its allies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Conidiogenous cells</th>
<th>Conidia (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. caledonia</td>
<td>ellipsoidal to conidial</td>
<td>ellipsoidal to more or less cylindrical, (2.4−)3.0−5.0(−6.5) × 1.0−1.8(−2.0)</td>
</tr>
<tr>
<td>B. gryllotalpidicola</td>
<td>flask-shaped</td>
<td>globose, 2 × 2</td>
</tr>
<tr>
<td>B. lii</td>
<td>ellipsoidal to cylindrical</td>
<td>ellipsoidal to cylindrical, (3.1−)4.3−6.5(−10.1) × (1.4−)2.1−2.6(−3.6)</td>
</tr>
<tr>
<td>B. loeiensis</td>
<td>cylindrical or narrowing at the tip</td>
<td>ellipsoidal to cylindrical, 3.5−6 × 1.5−2</td>
</tr>
<tr>
<td>B. medogensis</td>
<td>sub-spherical to flask-shaped</td>
<td>globose to subglobose, 2.0−3.0 × 2.0−3.5</td>
</tr>
<tr>
<td>B. rudraprayagi</td>
<td>sub-spherical to ampulliform</td>
<td>globose, subglobose, 2.5−4.0 × 2.5−4.0</td>
</tr>
<tr>
<td>B. majiangensis</td>
<td>cylindrical or sometimes sub spherical</td>
<td>ellipsoidal, 2.4−3.8 × 1.6−2.3</td>
</tr>
</tbody>
</table>

The nuclear ribosomal ITS and TEF were first used to identify cryptic diversification among Beauveria spp. by Rehner & Buckley (2005). Rehner et al. (2011) proposed a multilocus phylogeny of Beauveria species based on partial RPB1, RPB2, TEF, and Bloc sequences, and noted that each of the four loci used to reconstruct the Beauveria phylogeny could be individually used for accurate placement of all species, based on multiple species-specific phylogenetically informative nucleotide characters. In this study, the concatenated TEF+RPB1+Bloc and ITS analyses produced maximum likelihood and Bayesian trees that were largely congruent. Most branches were strongly supported in both analyses. The two B. majiangensis strains clustered together and were distinct from other Beauveria species. Thus, molecular phylogenetic results supported the morphologically based conclusion that strain GZU12141 is a new Beauveria species.

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