Journal of Invertebrate Pathology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Metarhizium alvesii sp. nov.: A new member of the *Metarhizium anisopliae* species complex

Rogerio B. Lopes[a], Daniela A. Souza[a], Luiz F.N. Rocha[b,c], Cristian Montalva[d], Christian Luz[c], Richard A. Humber[e], Marcos Faria[a,*]

[a] Embrapa Genetic Resources and Biotechnology, CP 02372, Brasília, DF 70770-917, Brazil
[b] Instituto Federal de Educação, Ciência e Tecnologia de Goiás, Aparecida de Goiânia, GO 74968-755, Brazil
[c] Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO 74690-900, Brazil
[d] Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Casilla 567, Universidad Austral de Chile, Valdivia 5090000, Chile
[e] USDA-ARS Emerging Pests and Pathogens Research Unit, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA

ARTICLE INFO

Keywords:
Ascomycota
Clavicipitaceae
Entomopathogenic fungi
Taxonomy
Multilocus phylogeny

ABSTRACT

A strain within the *Metarhizium anisopliae* species complex was isolated in 2009 from a soil sample in a banana plantation in the municipality of Quixeré, Northeastern region of Brazil. Previous studies showed that this insect-pathogenic strain does not fit with any current taxon within the *M. anisopliae* species complex, as determined by both genomic and by mass spectrometric analyses. In the present study, CG1123 (=ARSEF 13308) is shown to be morphologically indistinguishable from most species in this cosmopolitan species complex, whereas multilocus phylogeny confirmed its uniqueness and supports its recognition as a new species, *Metarhizium alvesii*, in honor of Sérgio Batista Alves, one of the founders of insect pathology in Brazil.

1. Introduction

The genus *Metarhizium* Sorokin (Hypocreales: Clavicipitaceae) includes well-known entomopathogenic fungi that serve as the active ingredients of over 60 commercial mycoinsecticides worldwide (Faria and Wraight, 2007). Multilocus phylogenetic analyses have clarified relationships within this genus and, although morphological differences are not always evident among species, well-justified proposals of new taxa have been made (Bischoff et al., 2009; Kepler et al., 2014; Montalva et al., 2016). Recent taxonomic refinements that focused on the cryptic diversity within the *M. anisopliae* species complex using multigene phylogeny have, so far, recognized 10 species in this group (Bischoff et al., 2009; Kepler et al., 2014).

With the exceptions of *M. globosum*, *M. guizhouense*, and *M. indigoticum*, the remaining species currently treated within the *M. anisopliae* species complex are noted to occur in Brazil: *M. anisopliae* sensu stricto (Faria et al., 2009; Rocha et al., 2013; Lopes et al., 2013b, 2014; Rezende et al., 2015), *M. pingshaense* (Xavier-Santos et al., 2011; Lopes et al., 2014), *M. robertsii* (Bischoff et al., 2009; Xavier-Santos et al., 2011; Rocha et al., 2013; Lopes et al., 2013b, 2014; Rezende et al., 2015), *M. brunneum* (Lopes et al., 2012, 2014), *M. lepidiotae* (Lopes et al., 2013b, 2014), and *M. acridum* (Magalhães et al., 1997; Driver et al., 2000; Lopes et al., 2014). Additionally, two taxonomically unassigned lineages within the *M. anisopliae* species complex have been reported (Rocha et al., 2013; Rezende et al., 2015). To our knowledge, other four *Metarhizium* species are known from Brazil: *M. pemphigi* (Rocha et al., 2013) and *M. blattodeae* (Montalva et al., 2016) from the *M. flavoviride* species complex; and *M. brasiliense* (Kepler et al., 2014), which is closely related to *M. album*, previously treated as *M. flavoviride* "Type E" by Driver et al. (2000). Finally, one of Brazil's most prominent fungal entomopathogens has been regarded to be *Metarhizium* (=*Nomuraea*) *rileyi* (Alves et al., 1978; Sujii et al., 2002).

One single *Metarhizium* sp. strain, CG1123, with no clear genomic relationship to any described species within the *M. anisopliae* species complex was obtained at a site located in a tropical zone with dry summer, according to Köppen's climate classification system (Alvares et al., 2013). The previous work also both confirmed the pathogenicity of this strain for the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) and provided a preliminary genomic assessment (Lopes et al., 2013a). A complementary study using mass spectrometry analyses confirmed the uniqueness of this strain as distinct from any other described species of *Metarhizium* (Lopes et al., 2014). In the present study, we characterized the morphology of CG1123 and conducted more extensive multigenic phylogenetic evaluations with this strain in order to evaluate whether this taxon should be recognized as a new species.

* Corresponding author.
E-mail address: marcos.faria@embrapa.br (M. Faria).

https://doi.org/10.1016/j.jip.2017.12.001
Received 20 March 2017; Received in revised form 4 December 2017; Accepted 6 December 2017

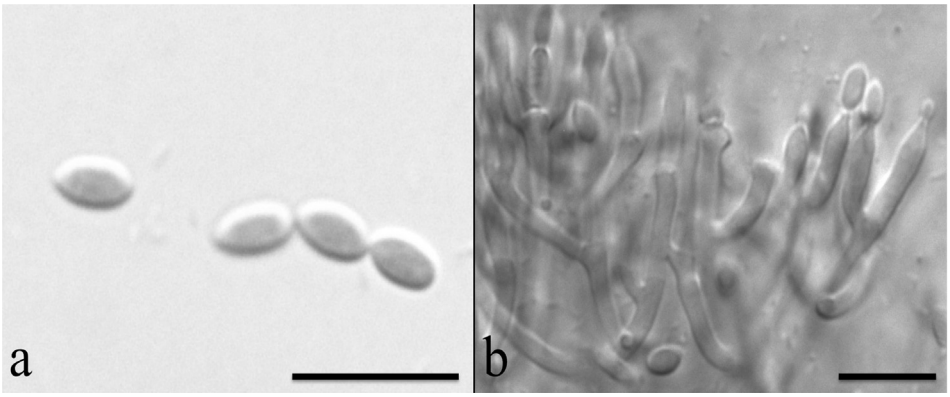

Fig. 1. Conidiogenous cells and conidia of *Metarhizium alvesii*. a. Conidia; b. Phialides with developing conidia. Bar = 10 µm.

2. Materials and methods

2.1. Field location, collecting and processing of material

Strain CG1123 was found in February 2009 in an intensively cultivated banana plantation in the municipality of Quixeré, Ceará state, Northeastern Brazil (Lopes et al., 2013a). It was isolated from a soil sample and maintained as a monosporic culture. Partial sequences of the genes β-tubulin (BTUB), RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2) and translation elongation factor 1-alpha (3′ end of the TEF-1α) were deposited in the GenBank under the accession numbers KY007611, KY007612, KY007613, and KY007614, respectively.

2.2. Morphological evaluations

This strain was investigated based on morphological characteristics, and semi-permanent slide mounts were prepared in lactophenol-cotton blue. The strain was grown on quarter-strength Sabouraud dextrose agar + yeast extract (SDAY/4: 2.5 g L^{-1} peptone, 10 g L^{-1} dextrose, 2.5 g L^{-1} yeast extract, 20 g L^{-1} agar) for 5–7 days in the dark at 25 ± 1 °C. Fungal microstructures (conidiophores, conidiogenous cells, and conidia) were examined by brightfield or phase contrast microscopy (Nikon Eclipse E600), documented with a Nikon DS-Fi1 digital camera, and measured with Motic Images Plus 2.0 software. Measurements were based on 50 objects per microstructure from which we calculated mean values, standard error of the mean (± SEM). The color of the conidial mass was determined using the Pantone color system (Eiseman and Herbert, 1990) as well a close approximation of this color for a computer monitor using the values of the CMYK (Cyan, Magenta, Yellow, black) color system and an adjustment for color opacity.

2.3. Molecular characterization

Strain CG1123 was grown in 150 mL on quarter strength SDY broth (SDY/4; 2.5 g bacto peptone, 10 g dextrose, 2.5 g yeast extract) for 7 days in a shaker at 125 rpm and 25 ± 1 °C. Hyphae and conidia were harvested, dried, and ground into a powder in liquid nitrogen with a mortar and pestle. A SDS (sodium dodecyl sulfate)-based method described by Raeder and Broda (1985) was adopted for DNA extraction. Partial sequences of the following four genes were amplified by polymerase chain reaction (PCR): BTUB using the primers BT1F and BT1R (Bischoff et al., 2009); RPB1 with RPB1C and RPB1Af (Stiller and Hall, 1997); RPB2 with fRPB2-5F and RPB2-7cR (Liu et al., 1999); and 3′ end of the TEF-1α with primers 983F and 2218R (Rehner and Buckley, 2005). The 5′ end of TEF-1α was previously sequenced by Lopes et al. (2013a). The PCR products were checked using agarose gel electrophoresis and sent for purification and sequencing by Helixxa Genomic Services (Paulínia, SP, Brazil). Both strands of the PCR products were sequenced using the Applied Biosystems Big Dye v.3.1 kit and the same primers described above with an ABI 3500 automatic sequencer. Contigs of CG1123 sequence data were assembled using Chromas Pro (V. 1.5, Technelysium Pty Ltd). Reference sequences were obtained from GenBank and are listed in Table 1 (see Supplementary Material). Multiple sequence alignments of each gene were made with Mega 5.0.3 by ClustalW and manually adjusted. The program jModelTest 0.1.1 (Posada, 2008) was used to identify the best-fit models of nucleotide substitutions using the corrected Akaike information criteria. A concatenated alignment (3′TEF, 5′TEF, RPB1, RPB2 and BTUB) was generated with Mesquite 3.04 software (Maddison and Maddison, 2015). Analyses of the consensus sequences of each single gene and the concatenated alignment were carried out under the Maximum Parsimony (MP) method, and bootstrap support (BS) values were provided. Additionally, we used Bayesian phylogenetic inference by MrBayes v. 3.2.1 (Ronquist et al., 2012), and BS values were included in the Bayesian trees. Analysis was run over ten million generations, with tree sampling every 100 generations; the first 25% of trees were discarded prior to consensus tree calculation.

3. Results

3.1. Taxonomy

Metarhizium alvesii Lopes, Faria, Montalva & Humber sp. nov. (Fig. 1).

MycoBank MB819472.

The colonies on SDAY/4 were initially colourless, becoming increasingly yellow immediately below developing conidial hymenia (typically after 5–8 days) and then greenish as conidia matured with the conidial mass bluish olive (Pantone 18-0316; CMYK 63:41:77:18). Conidiogenous cells ovoid to broadly ellipsoid, 10.91 ± 0.24 × 2.12 ± 0.03 µm (overall range: 7.55–14.46 × 1.60–2.80 µm), narrowed apically but without any obvious neck. Conidia cylindrical, 4.98 ± 0.07 × 2.63 ± 0.03 µm (overall range: 3.88–6.55 × 2.16–3.25 µm) (Fig. 1). *M. alvesii* cannot be distinguished from most related taxa based only on conidial and phialidic morphologies.

Holotype: UFG 50750 is a dried culture of CG1123 deposited in the Herbariun of the Federal University of Goiás, Goiânia, Brazil.

Ex-Type culture: CG1123, Invertebrate-Associated Fungal Collection (CFI), at Embrapa Genetic Resources and Biotechnology (Brasilia DF, Brazil), collected by R.B. Lopes, 02 February 2009, and co-deposited as ARSEF 13308 in the USDA-ARS Collection of Entomopathogenic Fungal Cultures (Ithaca, New York, USA).

Type locality: Commercial banana plantation at Quixeré, Ceará State, Brazil; S 05°09′10.5″, W 38°00′05.2″, at ca. 145 m above sea level.

Type substrate: soil sample from a commercial plantation of banana (*Musa* sp., genomic group AAB, cv. Prata-Anã) cultivated for 10 years and submitted to an intensive agricultural regime, including irrigation