Journal of Invertebrate Pathology 140 (2016) 46–50

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Short Communication

Phylogenetic structure and habitat associations of *Beauveria* species isolated from soils in Slovakia

Juraj Medo [a,*], Jaroslav Michalko [b,d], Janka Medová [c], Ľudovít Cagáň [a]

[a] Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[b] Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademická 2, 950 07 Nitra, Slovakia
[c] Constantine the Philosopher University in Nitra, Tr. A. Hlinku 1, 949 74 Nitra, Slovakia
[d] Institute of Forest Ecology, Arboretum Mlynany, Slovak Academy of Sciences, Vieska nad Zitavou 178, 951 52 Slepcany, Slovakia

ARTICLE INFO

Article history:
Received 22 March 2016
Revised 15 July 2016
Accepted 17 August 2016
Available online 18 August 2016

Keywords:
Phylogenetic structure
Beauveria spp.
Entomopathogenic fungi
Habitat preference

ABSTRACT

The phylogenetic structure of 109 soil-borne entomopathogenic Beauveria isolates acquired using the *Galleria mellonella* bait method from different habitat types in Slovakia was determined by sequence analysis of their ITS and Bloc loci. Three *Beauveria* species were identified; *Beauveria bassiana*, *B. pseudobassiana* and *B. brongniartii*, represented by 51.4%, 43.1% and 5.5% of acquired isolates, respectively, which were resolved into 15, 1 and 1 distinguishable haplotypes. Correlation analysis with the habitat type and individual habitat characteristics showed strong preferences of the most prevalent haplotypes for agricultural (*B. bassiana* A1) and forest habitats (*B. pseudobassiana*) which has possible implications for conservative biocontrol strategies.

1. Introduction

Anamorphic entomopathogenic fungi of the genus *Beauveria* belong to the most commonly isolated facultative pathogens of insect pests (Chandler et al., 1997; Bidochka et al., 1998; Vega et al., 2012). A high level of genetic diversity is commonly detected within natural *Beauveria* populations. General phylogenetic markers such as intergenic spacer (ITS) or elongation factor 1-α are suitable for distinguishing between *Beauveria* species but are not able to characterize this diversity in deep. Recently developed highly variable markers, specifically the Bloc marker (a nuclear intergenic marker developed for phylogenetic investigation of *Beauveria*) as well as microsatellites enable further resolution of *Beauveria* isolates at the level of individual genotypes (Coates et al., 2002; Meyling and Eilenberg, 2006; Castrillo et al., 2007; Hollingsworth et al., 2011). Phylogenetic studies using these markers have shown that diversity within the *Beauveria* genus could be both on inter- and intra-specific level. For example, it has been found that *B. bassiana sensu lato* and *B. brongniartii* represent a complex of morphologically cryptic phylogenetic species (Rehner, 2005; Rehner et al., 2006), which led to a revision of *Beauveria* taxonomy. Three morphologically similar phylogenetic lineages within the *B. bassiana* complex were reclassified as *B. pseudobassiana* (previously designated as Clade C), *B. varroae* and *B. kipukae*. Additionally, two sister lineages within the *B. brongniartii* complex were reclassified as *B. asiatica* and *B. australis*, which increased the number of currently known *Beauveria* species to twelve (Rehner et al., 2011). To distinguish between the remaining thus far unresolved lineages within the *B. bassiana* complex a temporary annotation system has been proposed, which designates lineages based on the continent from which they were isolated followed by a digit indicating the order of their discovery, e.g. Eu_1 (Meyling et al., 2009). However, many detected lineages have a worldwide distribution; therefore, an *ad hoc* naming system based on Rehner and Buckley (2005) without indication of continent should be preferred (e.g. A1), and a reference isolate for each lineage should be deposited in a culture collection (Stephen A. Rehner, personal communication).

Soil is the most commonly examined environment when assessing *Beauveria* diversity. Baiting techniques using susceptible larvae of *Galleria mellonella* (Lepidoptera: Pyralidae), commonly referred to as the "*Galleria* bait method" (GBM), or *Tenebrio molitor* (Coleoptera: Tenebioidae) are most widely used for isolation of soil-borne *Beauveria* fungi, although the use of selective media or a combination of both methods may improve the isolation efficacy (Meyling et al., 2009; Medo and Cagáň, 2011). Three species of *Beauveria* are commonly detected using these methods: *B. bassiana*, *B. pseudobassiana*, and *B. brongniartii*. Using the GBM *B. bassiana* A1 was found by Pérez-González et al. (2014) to be the most common

* Corresponding author.
E-mail address: juraj.medo@uniag.sk (J. Medo).

http://dx.doi.org/10.1016/j.jip.2016.08.009
0022-2011/

genotype in Mexican agricultural soils. The GBM was also used by Sevim et al. (2010) to find that *B. pseudobassiana* and *B. bassiana* A1 and A11 were the most abundant lineages in Turkey. Meyling et al. (2009), using baiting techniques as well as selective media for isolation of fungi from soil, detected lineages A1 (labeled as Eu_1), A3 (Eu_3) and A11 (Eu_6) in their study in Denmark. Lineages A6 (named as Wd-2) and A3 (Eu_3) were also identified by Garrido-Jurado et al. (2011), who analyzed the EF1-α region of Spanish *B. bassiana* isolates from culture collection. Additionally, lineages Eu_8 and Eu_9 were reported by these authors. However, Rehner and Buckley (2005) reported much higher diversity of *Beauveria* isolates from culture collections collected throughout Europe.

Despite improvements made in isolation, taxonomy, and genotyping of *Beauveria* fungi, major factors determining their genotypic structure at the population level remain poorly understood. Previous studies have linked a particular genetic group of the fungus with its geographical origin (Reay et al., 2010; Sevim et al., 2010; Meyling et al., 2012b; Cai et al., 2013), although some genetic groups have a world-wide distribution. A large number of studies have tried to find an association between a particular genetic group and its insect host (Maurer et al., 1997; Rehner et al., 2006; Reay et al., 2008; Meyling et al., 2012a; Wang et al., 2013). However, only limited evidence has been found to support this association and the general conclusion is that genetic groups in *Beauveria* spp. do not have any particular host preferences (Meyling et al., 2009). Certain studies have proposed that habitat-related environmental factors constitute the prime selective forces shaping the genetic structure of facultative entomopathogenic fungi (Vanninen, 1996; Bidochka et al., 1998, 2001, 2002; Meyling and Eilenberg, 2007; Quesada-Moraga et al., 2007; Meyling et al., 2009; Medo and Cagáň, 2011). Despite the existing consensus, few studies have investigated this hypothesis (Bidochka et al., 2002; Meyling et al., 2009). In our study we addressed this question by phylogenetic and correlation analysis of a subset of *Beauveria* soil isolates from different habitat types across Slovakia isolated using the GBM.

2. Material and methods

2.1. Soil sampling and isolation of fungi

Soil samples were collected from various locations within Slovakia during the year 2008 (Fig. 1A, Supplementary Table S1) and processed as described in Medo and Cagáň (2011). The sampling sites were selected with the aim of maximizing the spectrum of environmental conditions covered (altitude, soil characteristics, vegetation type). The minimum distance between the adjacent sampling sites was 1 km. For each soil sample the habitat type (forest, field, meadow or hedgerow), altitude of the sampling site, type of the vegetation canopy, and basic soil characteristics (pH, texture) were recorded (Supplementary Table S1). *Beauveria* fungi were isolated from soil samples using the *Galleria mellonella* baiting technique as described previously (Medo and Cagáň, 2011) and cultured on Sabouraud dextrose agar (SDA) (Merck KGaA).

2.2. Genetic analysis

DNA was extracted from two-week old single spore fungal cultures using the ZR fungal/bacterial DNA extraction kit (Zymo Research Corp. USA). All PCR reaction mixtures contained 200 mM dNTPs, 1x DreamTaq buffer, 0.5 unit DreamTaq DNA polymerase (Life technologies, USA), 0.5 mM of the corresponding primer, and 0.5 µl of non-diluted DNA. Cycling conditions were as follows: 95 °C for 3 min followed by 35 cycles of 95 °C for 30 s, annealing at corresponding temperature for each primer pair for 45 s, 72 °C for 90 s, and a final elongation at 72 °C for 10 min. Primers used for PCR and sequencing of ITS region were ITS1 and ITS4 (White et al., 1990). Primers 5.1F and B822Ldg were used for *B. pseudobassiana* and 5.1F and 3.1R for other *Beauveria* isolates to amplify the nuclear intergenic region Bloc. Amplification products were sequenced with primers B822Ldg and B22Udg (Rehner et al., 2011).

Sequences were analyzed in an ABI310 genetic analyzer using BigDye 3.1 sequencing chemistry (Life technologies, USA). Acquired sequences were assembled and processed using the Seaview software (Gouy et al., 2010). Alignment was made using MUSCLE (Edgar, 2004). The acquired unique haplotype sequences as well as the reference sequences (for Genbank accessions see Supplementary Table S2) were used for phylogenetic analysis. A phylogenetic tree was constructed using Phy ML (Guindon et al., 2010) with a GTR substitution model, a BioNJ starting tree with the best of NNI and SPR tree searching, and followed by 1000 bootstrap replications.

2.3. Statistical analyses

The presence/absence of certain genetic groups between two habitats was evaluated using cross-tabulation followed by the Fisher exact test and the odds ratio was then calculated. The occurrence of genetic groups at different soil pH's and altitudes was evaluated using the analysis of variance along with the Tukey HSD test. These statistics were only computed for *Beauveria* groups with a sufficient number of representatives, i.e. *B. pseudobassiana* and *B. bassiana* clade A1. All statistical analyses were performed using the Statgraphics XV package (StatPoint Technologies Inc. USA). The estimates of haplotypic diversity were calculated according to Stoddart and Taylor (1988) and expressed as the index of haplotypic diversity (G).

3. Results and discussion

Maximum likelihood analysis of the combined ITS and Bloc data resolved 109 analyzed *Beauveria* isolates into 8 phylogenetic clades (Fig. 1B) represented by three *Beauveria* species: *B. bassiana*, *B. pseudobassiana* and *B. brongniartii* containing 56 (54.1%), 47 (43.1%) and 6 (5.5%) isolates, respectively. Isolates of *B. pseudobassiana* and *B. brongniartii* were each represented by a single phylogenetic clade and haplotype while isolates of *B. bassiana* were resolved into six distinct phylogenetic clades (A1, A2, A3, A6, A11 and AX containing respectively 42, 2, 9, 1, 1 and 1 isolates) comprising 15 fungal haplotypes (Fig. 1B).

The recognized species composition of *Beauveria* fungi in Slovakian soils is similar to that reported by other authors in and outside of Europe (Meyling et al., 2009; Sevim et al., 2010; Garrido-Jurado et al., 2011; Pérez-González et al., 2014; Mayerhofer et al., 2015). The genotypic composition of *Beauveria* spp., on the other side, varies among studies from different geographic areas. Although the comparison of results among studies is not straightforward due to differences in employed protocols for fungal isolation and different genetic markers for isolate genotyping, the composition of main *Beauveria* lineages appears to be relatively stable across Europe with *B. bassiana* A1 and *B. pseudobassiana* being the predominant lineages. Whether this indicates that the less frequent lineages are worse competitors in the soil environment or that their occurrence is restricted to specific environmental conditions remains to be determined.

Within individual habitats, we found that the haplotypic diversity (G) of *Beauveria* isolates was highest in field soils ($G = 5.39$) and lowest in forests soils ($G = 1.53$). Only three (A1g, A3b and AX) out