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Original article

Assessment of the genetic diversity of Argentinean isolates of *Beauveria bassiana* (Ascomycota: Hypocreales) using ISSR markers

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ABSTRACT

Recombination mediated through a parasexual cycle is a potential source of genetic diversity among mitosporic fungi such as *Beauveria bassiana*. Knowledge of this diversity is critical to understand the structure of the population. Therefore, the aim of this study was to analyze the genetic diversity within 36 Argentinean isolates of *B. bassiana* collected from different geographical regions and insect hosts; and to assess by means of ITS sequences the preliminary taxonomic position of the isolates that might be related to the phenotypic and genotypic differences. The results demonstrate that the fingerprints generated with four ISSR markers were enough to detect significant genetic diversity among Argentinean isolates, which was unrelated neither with the geographical origin nor with insect hosts.

1. Introduction

Beauveria bassiana (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales) is one of the most commonly used fungal pathogens in biological control of insect pests worldwide; mainly due to its wide host range, which includes more than 700 species of insects (Rehner, 2005). It is a cosmopolitan, haploid and mitosporic organism that, in addition to its saprotrophic habit in a wide array of environments such as the soil, barks and tree foliage (Ormond et al., 2010) it behaves as an endophyte of several cultivated plants (Lewis et al., 2001; Vega, 2008; White et al., 2002).

It is currently accepted that *B. bassiana* forms a complex of several cryptic species that are phylogenetically positioned within the family Cordycipitaceae belonging to the order Hypocreales (Rehner and Buckley, 2005). Morphologically, *Beauveria* is characterized by its sympodial to whorled clusters of short, globose to flask-shaped conidiogenous cells, which give rise to a succession of one-celled, hyaline, holoblastic conidia borned on a progressively elongating sympodial rachis. Although the morphological characterization allows identification of the genus, species recognition is difficult because of its structural simplicity, the lack of distinctive phenotypic variations and the great intraspecific diversity that is manifested at the phenotypic and genotypic levels, mainly within *B. bassiana* (Burdon and Silk, 1997; Dalzoto et al., 2003; Fernandes et al., 2006; Gaitan et al., 2002; Garrido-Jurado et al., 2011; Rehner and Buckley, 2005; St Leger et al., 1992; Takatsuka, 2007; Toledo et al., 2008). In addition, identification of representatives of *Beauveria* has increased in complexity since new species were described between the end of the 19th century and the mid 20th century; which led to identify new tools that might complement conventional taxonomy based on morphological characters. Rehner and Buckley (2005) and Rehner et al. (2011) conducted a phylogenetic analysis with nucleotide sequences of the ribosomal internal transcribed spacers (ITS), nuclear intergenic region Bloc, translation elongation factor 1-alpha (EF1-α), RNA polymerase II largest subunit (RPB1) and RNA polymerase II second largest subunit (RPB2). The clades generated from these studies corresponded with the species of *Beauveria* previously defined on the basis of conidia and colony morphology. These results showed that the combination of morphological characters with molecular markers is critical to perform a precise identification of the species within the genus *Beauveria*.

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Several studies have been performed using PCR molecular markers with the aim of identifying polymorphisms within the genome of *Beauveria* (Aquino de Muro et al., 2003; Castrillo et al., 2003; Coates et al., 2002; Enkerli et al., 2001; Glare and Inwood, 1998; Maurer et al., 1997; Wang et al., 2003). In the last decade inter-simple sequence repeats (ISSR) has been successfully applied to diversity studies. These are markers of high reproducibility, which reveal a large number of information bands in a single amplification, thus being one of the most commonly used markers in intraspecific differentiation and highly effective for the identification of genotypes of *B. bassiana* (Aquino de Muro et al., 2005; Estrada et al., 2007; Fontecha et al. 2011; Ormond et al., 2010; Wang et al., 2013).

Knowledge of the genetic diversity and intraspecific relationships within a collection of isolates is critical to understand the structure of the population. Therefore, the aim of this work was to analyze by means of ISSR markers the genetic diversity of 36 isolates of *B. bassiana* associated to several insect hosts and geographical regions of Argentina and also assess the taxonomic position of the isolates by means of ITS sequences.

2. Materials and methods

2.1. Origin of biological material

Isolates of *B. bassiana* were obtained from infected insects of the orders Hemiptera, Coleoptera and Dermaptera and from soil samples of agricultural crops, collected in several localities of Buenos Aires, Corrientes and Tucumán provinces that correspond to three different agro-ecological regions (Pampas, Northeast and Northwest, respectively) (Toledo et al., 2008). Cultures were preserved in the mycological collections of Centro de Estudios Parasitológicos y de Vectores- CEPAVE (La Plata, Buenos Aires, Argentina) and the USDA-Agricultural Research Service Collection of Entomopathogenic Fungal Cultures-ARSEF (Ithaca, New York, USA), where they were admitted under their respective accession numbers (Table 1).

2.2. DNA extraction

DNA was extracted from fungal cultures developed in 2% Malt Extract Agar (MEA) medium after 7 d incubation at 25 °C in darkness (Zhang et al., 2010). The quality of genomic DNA was assessed by electrophoresis on 0.7% w/v agarose gels supplemented with ethidium bromide (100 ng ml^{-1} final concentration). The amount of DNA was estimated by comparison with a molecular marker control of known concentration (Lambda Phage Genome digested with Hind III – Promega Biotech) using the image analyzer (SYNGENE GeneTools).

2.3. Diversity analysis

After evaluating a total of eleven ISSR markers, 3′ anchored and 5′ anchored, four were selected to perform the analysis of 36 isolates of *B. bassiana* (Table 2). Amplification reactions including DNA free control were carried out in a 15 μL volume of reaction, containing 10× reaction buffer (500 mM KCl; 100 mM Tris–HCl, pH 9.0 at 25 °C; 1% Triton X-100), 2 mM (primers KA5 and 826) or 2.5 mM (primers BA3 and CA5) of $MgCl_2$, 0.25 mM each dNTP, 60 ng of each primer, 1 U Taq DNA polymerase and 100 ng of template DNA (reagents Inbio Highway®, Tandil, Buenos Aires, Argentina). PCR reactions were performed on a MJ Research (PTC-150 MiniCycler) programmed as follows: initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 48 °C for 45 s and extension at 72 °C for 3 min, with an additional final extension step at 72 °C for 5 min.

PCR products were resolved by electrophoresis on 1.5% w/v agarose gels supplemented with ethidium bromide (100 ng ml^{-1} final concentration) using as an internal standard the DNA marker 100–1000 bp (Inbio Highway®, Tandil, Buenos Aires, Argentina) and photographed. The ISSR banding patterns generated were analyzed with the SYNGENE GeneTools software and a dendrogram was built using the similarity matrix of DICE and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), with a 5% tolerance.

2.4. Molecular identification

The identity of the isolates of *B. bassiana* previously characterized on the basis of morphological characters (Toledo et al., 2007, 2008) was confirmed by the amplification of the ITS, which was performed on seven of the 36 isolate. They were selected on the basis of their phenotypic differences, geographical origin, host and location in the dendrogram generated through the analysis of diversity. Universal primers ITS5 (5′-GGAAGTAAAAGTCGTAA CAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were used for the isolates Bb147 and Bb189 and ITS1 (5′-TCCGTAGGT GAACCTGCGG-3′) and ITS4 for the remaining isolates (White et al., 1990). The PCR program consisted in an initial cycle of denaturation at 94 °C for 4 min; followed by 33 cycles of denaturation at 94 °C for 1 min, 45 s at 56 °C, 1 min at 72 °C and a final elongation step at 72 °C for 5 min. The reaction mixture contained 1× reaction buffer (500 mM KCl; 100 mM Tris–HCl, pH 9.0 at 25 °C; 1% Triton X-100 without magnesium); 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.3 μM each primer; 1U of T-Plus DNA Polymerase (Highway Molecular Biology-INBIO-UNICEN) and 50 ng of template DNA per reaction, in a final volume of 15 μL. Amplification products were resolved by electrophoresis on a 1% w/v agarose gel supplemented with ethidium bromide (100 ng ml^{-1} final concentration).

Amplified fragments were precipitated by adding a volume of isopropanol and 0.1 volumes of sodium acetate 3 M. The mixture was incubated at −18 °C for 12 h and then centrifuged at 15,000*g* for 15 min. DNA pellet was washed with ethanol 70%, subsequently to be dried and dissolved in sterile distilled water. Amplicons were sequenced at Macrogen Inc. (Seoul, Korea) according to the method described by Sanger et al. (1977). Sequences were edited using the program BioEdit version 7.0.9.0 (Hall, 1999) and they were used to perform a phylogenetic analysis that included the sequences of the isolates of *Beauveria* from this work with sequences of some species for the genus available at the GenBank database (www.ncbi.nlm.nih.gov). Two representatives of each species of *B. bassiana* (AY531984.1 and NR_111594.1, the last one designated as neotype by Rehner et al. (2011), *B. brongniartii* (HQ880770.1 and DQ376245.1), *B. amorpha* (HQ880804.1 and HQ880806.1), *B. caledonica* (HQ880818.1 and HQ880821.1), and *B. malawiensis* (DQ376247.1 and HQ880825.1) were used as in-group, while the sequence of a representative of *Lecanicillium saksenae* (AB360351.1) was used as out-group. Sequences were aligned with the ClustalW tool of the program Mega5 (Tamura et al., 2011), assuming a gap opening penalty of 15 and a gap extension penalty of 6.66. The alignment was edited automatically by the software Gblock Version 0.91b (Talavera and Castresana, 2007; Tamura et al., 2011), setting to 2 the minimum length of a block and leaving all the other options by default. The phylogenetic study was carried out using the Bayesian inference and analysis of maximum likelihood. To do this, the best substitution model was selected using the Akaike Information Criterion (AIC) (Akaike, 1973) in the jModelTest software version 2.1.7 (Ḍ'arriba et al., 2012), and

Table 1
List of *Beauveria bassiana* fungal isolates, mycological collections accession numbers, source of isolation and geographical origin.

Isolates[a]	Host/substrate	Geographical origin	Agro-ecological region
CEP001/ARSEF8368	Soil	Buenos Aires 35°05′29.4″ S – 57°30′41.5″ W	Pampas
CEP002/ARSEF8369	Soil	Buenos Aires 34°54′35.9″ S – 57°55′41.5″ W	Pampas
CEP061/ARSEF7463	Coleoptera: Curculionidae	Buenos Aires 35°0.1′49.5″ S – 58°26′56.9″ W	Pampas
CEP069/ARSEF8459	Hemiptera: Cicadellidae	Buenos Aires 34°56′19.2″ S – 58°0.6′3.8″ W	Pampas
CEP072/ARSEF7464	Hemiptera: Cicadellidae	Buenos Aires 34°56′19.2″ S – 58°0.6′3.8″ W	Pampas
CEP074/ARSEF7465	Hemiptera: Cicadellidae	Buenos Aires 34°51′ S – 58°06′ W	Pampas
CEP077/ARSEF7467	Hemiptera: Cicadellidae	Buenos Aires 34°57′29.5″ S – 58°5′26.6″ W	Pampas
CEP080/ARSEF7468	Hemiptera: Membracidae	Buenos Aires 35°0.1′49.5″ S – 58°26′56.9″ W	Pampas
CEP081/ARSEF7469	Hemiptera: Cicadellidae	Buenos Aires 35°0.1′49.5″ S – 58°26′56.9″ W	Pampas
CEP083/ARSEF7470	Coleoptera	Tucumán 26°47′33.4″ S – 65°19′05.3″ W	Northwest
CEP092/ARSEF7471	Coleoptera: Curculionidae	Buenos Aires 34°57′59″ S – 58°04′42″ W	Pampas
CEP111/ARSEF8460	Hemiptera: Cicadellidae	Buenos Aires 34°51′ S – 58°06′ W	Pampas
CEP112/ARSEF8520	Coleoptera: Curculionidae	Buenos Aires 34°57′59″ S – 58°04′42″ W	Pampas
CEP113/ARSEF8521	Coleoptera: Curculionidae	Buenos Aires 34°57′59″ S – 58°04′42″ W	Pampas
CEP116/ARSEF8523	Coleoptera: Curculionidae	Buenos Aires 34°57′59″ S – 58°04′42″ W	Pampas
CEP117/ARSEF8524	Coleoptera: Curculionidae	Buenos Aires 34°57′59″ S – 58°04′42″ W	Pampas
CEP118/ARSEF8525	Coleoptera: Curculionidae	Buenos Aires 34°57′59″ S – 58°04′42″ W	Pampas
CEP119/ARSEF8526	Coleoptera: Curculionidae	Buenos Aires 34°57′59″ S – 58°04′42″ W	Pampas
CEP137/ARSEF8370	Hemiptera: Cicadellidae	Buenos Aires 34°51′ S – 58°06′ W	Pampas
CEP138	Coleoptera: Curculionidae	Buenos Aires 35°05′29.4″ S – 57°30′41.5″ W	Pampas
CEP140/ARSEF8529	Hemiptera: Cicadellidae	Buenos Aires 35°05′29.4″ S – 57°30′41.5″ W	Pampas
CEP141/ARSEF8530	Coleoptera: Curculionidae	Buenos Aires 35°05′29.4″ S – 57°30′41.5″ W	Pampas
CEP142/ARSEF8371	Hemiptera: Cicadellidae	Buenos Aires 34°51′ S – 58°06′ W	Pampas
CEP143	Hemiptera: Cicadellidae	Buenos Aires 34°51′ S – 58°06′ W	Pampas
CEP146	Coleoptera	Buenos Aires 34°51′ S – 58°06′ W	Pampas
CEP147/ARSEF8372	Coleoptera: Coccinellidae	Tucumán 26°49′50.2″ S – 65°16′59.4″ W	Northwest
CEP148/ARSEF8462	Coleoptera: Coccinellidae	Tucumán 26°49′50.2″ S – 65°16′59.4″ W	Northwest
CEP149/ARSEF8463	Coleoptera: Coccinellidae	Tucumán 26°49′50.2″ S – 65°16′59.4″ W	Northwest
CEP150/ARSEF8373	Coleoptera: Chrysomelidae	Tucumán 26°49′50.2″ S – 65°16′59.4″ W	Northwest
CEP151/ARSEF8374	Dermaptera: Forficulidae	Tucumán 26°49′50.2″ S – 65°16′59.4″ W	Northwest
CEP153	Hemiptera: Cicadellidae	Buenos Aires 35°0.1′49.5″ S – 58°26′56.9″ W	Pampas
CEP175	Coleoptera: Lampyridae	Corrientes 29°11′43.5″ S – 58°02′0.8″ W	Northeast
CEP176	Coleoptera: Lampyridae	Corrientes 29°11′43.5″ S – 58°02′0.8″ W	Northeast
CEP189/ARSEF7776	Hemiptera: Cixiidae	Los Hornos, Buenos Aires 34°54′35.9″ S – 57°55′41.5″ W	Pampas
CEP249/ARSEF8465	Dermaptera: Forficulidae	Buenos Aires 34°38′ S – 60°28′ W	Pampas
Bb55[b]	Coleoptera: Chrysomelidae	Corrientes 28°59′ S – 59°06′ W	Northeast

[a] Abbreviations for collections: CEP: Centro de Estudios Parasitológicos y de Vectores, La Plata, Buenos Aires, Argentina; ARSEF, USDA-ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, New York, USA.
[b] Isolate belonging to the personal collection of the first author.

Table 2
Primers used to amplify ISSR regions along the genome of *Beauveria bassiana* and their respective oligonucleotide sequences.

Primer	Sequence	Primer	Sequence	Primer	Sequence
AA3	$(AG)_8TA$	IA5	$ACA(CAA)_5$	846	$(CA)_8RT$[a]
CA5	$CAT(ACA)_5$	KA5	$CT(AC)_8$	850	$(GT)_8YC$[b]
BA3	$(AC)_8CT$	826	$AC(CA)_6CC$	854	$(TC)_8RG$
EN	$(GAC)_5$	828	$(TG)_8A$		

[a] R = A, G.
[b] Y = C, T.

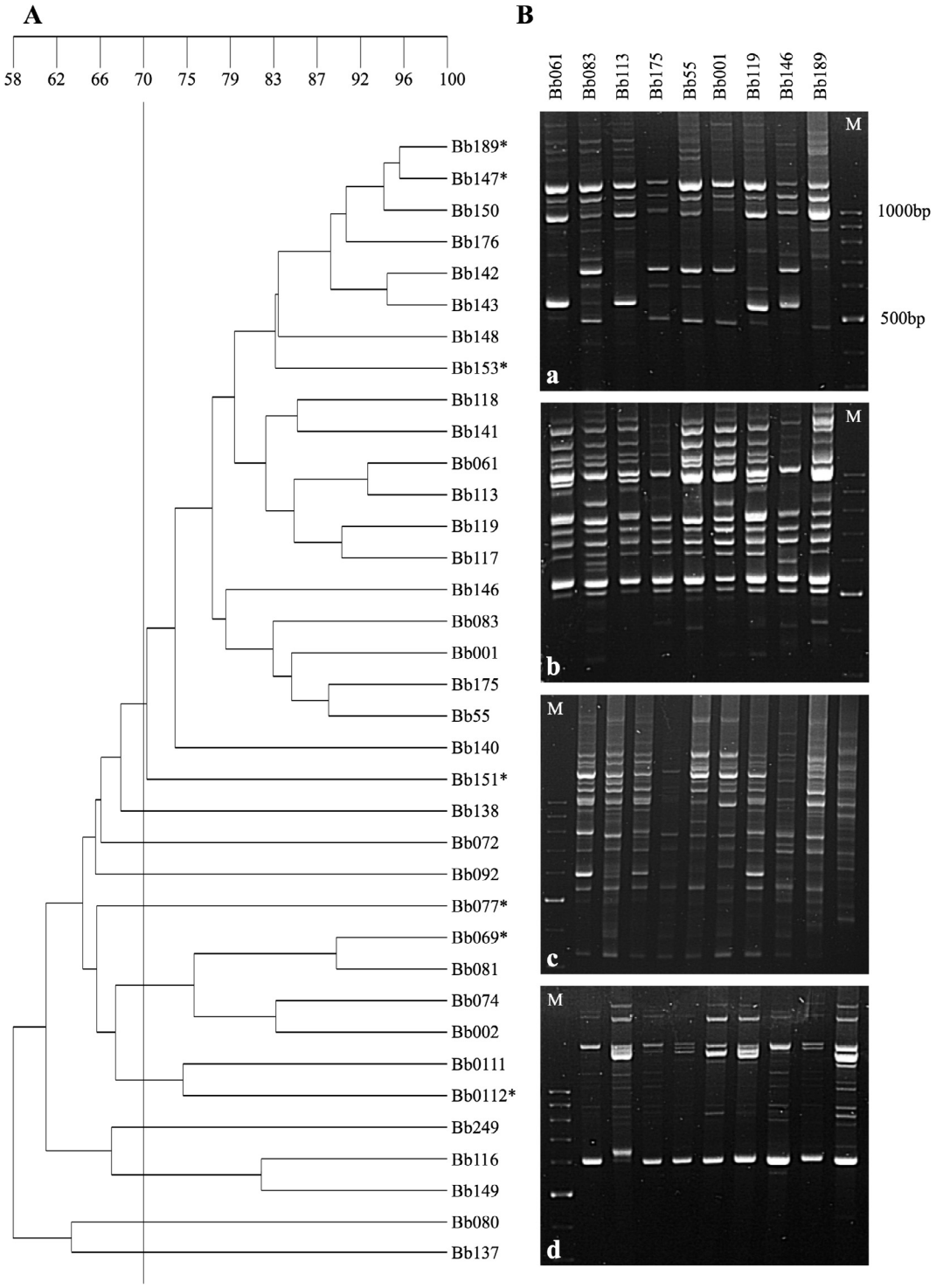

Fig. 1. (A) Dendrogram built using the similarity matrix of DICE and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) which includes 36 isolates of *Beauveria bassiana*; (B) ISSR amplification patterns of nine isolates (Bb061, Bb083, Bb113, Bb175, Bb55, Bb001, Bb119, Bb146 and Bb189) generated with the primers BA3 (a), CA5 (b), 826 (c) and KA5 (d). M = DNA marker 100–1000 bp. * Isolates selected for the specific identification from the sequencing of the ITS.

the parameters of the chosen model were used in the subsequent analysis. The maximum likelihood analysis was carried out with the PhyML software v.3.0 (Guindon and Gascuel, 2003) using the BioNJ algorithm to obtain starting trees whose topologies were then optimized by both nearest neighbor interchange (NNI) and sub-tree pruning and regrafting (SPR) algorithms. The statistical support for the nodes was evaluated through 1000 bootstrap replicates. For the Bayesian analysis, the software MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was used to perform two independent Metropolis-coupled Markov chain Monte Carlo (MCMCMC) with four chains through 1,000,000 generations, with trees in each chain sampled every 100th generation. The first 25% of trees were removed as burn-in and posterior probabilities were determined from the remaining trees.

3. Results

3.1. Diversity analysis

Primers 826, BA3, CA5 and KA5 (Table 2), selected on the basis of the number of bands amplified and their stability, generated a total of 101 fragments, 92 of which were polymorphic. Primers generated between 10 and 44 bands (namely, 826 = 10; BA3 = 20; CA5 = 27; KA5 = 44) whose sizes ranged from 300 to 1700 bp. The banding patterns generated by combining the fingerprints generated with each primer were analyzed by agreeing to take a 70% of similarity as court, and in this way, it was noted that each isolate had a different haplotype (Fig. 1).

3.2. Molecular identification

The ITS amplicons of seven representative isolates of *B. bassiana* had a size between 487 bp and 568 bp and their sequences were deposited in the databases DDBJ/EMBL/GenBank under the accession numbers KU702657.1 (Bb069), KU702658.1 (Bb077), KU702659.1 (Bb112), KF308683.1 (Bb147), KU702660.1 (Bb151), KU702661.1 (Bb153) and KT952326.1 (Bb189). From the alignment of the nucleotide sequences, the software Gblock selected 499 reliable characters to be used in the phylogenetic study. The best substitution model according to Akaike Information Criterion (AIC), was the General Time-Reversible model with Invariant sites (GTR + I). By incorporating the chosen substitution model of molecular evolution into the phylogenetic analysis, under the maximum likelihood approach in PhyML v.3.0 and Bayesian inference in MrBayes v.3.2, similar tree topologies were obtained. Fig. 2 shows the Bayesian phylogram, in which it is noted that all the isolates included in this study were grouped together with two reference isolates of *B. bassiana* (AY531984.1 and NR_111594.1 = neotype) with high supporting values (posterior probabilities = 1 and bootstrap = 99). Furthermore, *B. brongniartii*, *B. amorpha*, *B. caledonica* and *B. malawiensis*, all morphologically-related species, formed independent clades.

4. Discussion

This work demonstrates that the fingerprints generated with four ISSR markers were enough to determine the diversity of the 36 isolates of *B. bassiana* that were collected from several sites of Argentina. Estrada et al. (2007) and Fontecha et al. (2011) also found that one and three primers, respectively, were enough to generate a haplotype for each of 11 isolates of *B. bassiana*. Regarding the number of primers used, the level of diversity observed in this work among the 36 isolates of *B. bassiana*, was similar to the level of diversity reported by Wang et al. (2005), who used 18 primers to characterize the same number of isolates. These results suggest that the levels of diversity detected by ISSR

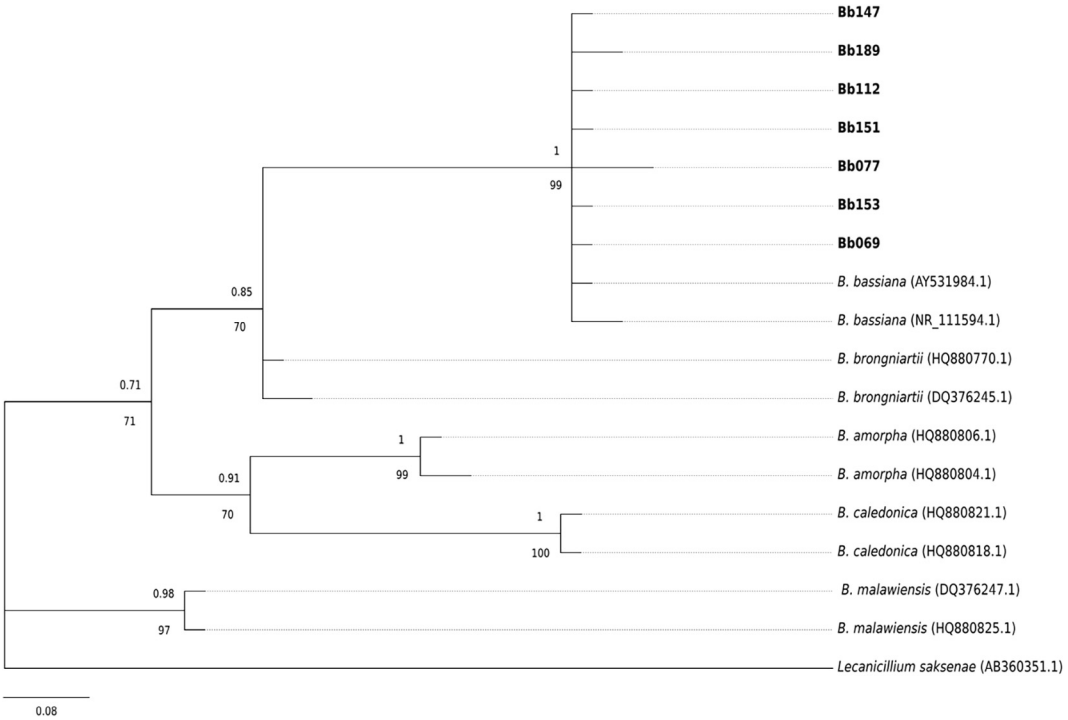

Fig. 2. Phylogenetic tree generated by the Bayesian analysis of the ITS sequences of seven Argentinean isolates of *Beauveria bassiana* and related species. *Lecanicillium saksenae* was used as out-group. The isolates corresponding to this work are highlighted in bold. Below each of the reference isolates detailed its number of access to GenBank. The probability values later ≥0.7 and bootstrap ≥70% are represented above and below of the internodes, respectively. The bar at the bottom indicates the number of substitutions per site.

will not be drastically augmented by increasing the number of primers.

The 36 isolates of *B. bassiana* analyzed in this study showed to be genetically diverse, which is in accordance with the previously observed phenotypic diversity, regarding colony morphology and synthesis of water-soluble pigments (Toledo et al., 2008). It is well known that in fungal populations the mitotic recombination mediated through a parasexual cycle is a potential source of genetic diversity (Burdon and Silk, 1997). Particularly in *B. bassiana*, Dalzoto et al. (2003) detected different alternative means of recombination as evidence of this diversity, which prevail between isolates that share a common ecological niche. Therefore, it seems reasonable to assume that the genetic diversity detected in the present study may be at least in part the result of such recombinations. It is also worth mentioning that the genetic diversity among strains bears no relation neither with the different agro-ecological regions from Argentina nor with insect hosts, which contrasts with the results obtained by Aquino de Muro et al. (2003), Estrada et al. (2007), Wang et al. (2005, 2013), who found that the diversity of *B. bassiana* was related to the geographical origin of the isolates. These incongruencies regarding the geographical stratification could be related to sample size, since this study was based on 36 isolates coming only from three Argentinean provinces within three agro-ecological regions, while the above-mentioned studies considered isolates from different countries.

On the other hand, it is interesting to mention that the analysis showed that highly virulent isolates against planthoppers pests of cereal crops (Toledo et al., 2007), such as Bb147, Bb150 and Bb189, were grouped within the same cluster with a level of similarity >90%. Future studies will confirm if the amplified bands that explain this grouping contain genes directly related to the virulence, or if they are close to the determinants of pathogenic activity.

The identification of the species of the genus *Beauveria* has traditionally been based on morphological characters; however, morphologically identical but genetically different isolates have been described, suggesting the existence of cryptic species (Rehner and Buckley, 2005). Currently, the taxonomic identification of species is not solely based on morphological characters, but molecular tools have been incorporated, allow to confirming the identity of the organisms. On this regard, the results from this work show that the analysis of ITS sequences along with the phenotypic characterization provide reliable information for the identification of species within the genus *Beauveria*. All isolates tested were placed together with those of reference within a same clade and separated from other morphologically-related species of *Beauveria*, beyond the phenotypic variation previously noted (Toledo et al., 2008), which is broadly in accordance to that observed by Rehner and Buckley (2005).

5. Conclusions

In conclusion, results of this study demonstrate that the fingerprints generated with four ISSR markers were enough to determine the diversity of the 36 isolates of *B. bassiana* providing valuable evidence for decision making in choosing of markers for future works. Accordingly, the analysis of the ITS sequences contributed to confirm the identity of the isolates. Moreover, the previously observed morphological diversity was reflected in the genetic diversity, which in the case of this mitosporic fungus might have arisen by recombination events within the genome. Finally, such diversity was not related neither with geographical origin of the strains nor with their insect hosts, which is to be expected due to the cosmopolitan nature and the broad host range of this fungus.

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Conflicts of interest

The authors have no conflict of interest to declare.

References

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov, B.N., Csaki, F. (Eds.), Second International Symposium on Information Theory. AkademiaiKiado, Budapest, pp. 267–281.

Aquino de Muro, M., Mehta, S., Moore, D., 2003. The use of amplified fragment length polymorphism for molecular analysis of *Beauveria bassiana* isolates from Kenya and other countries, and their correlation with host and geographical origin. FEMS Microbiol. Lett. 229, 249–257.

Aquino de Muro, M., Elliott, S., Moore, D., Parker, B.L., Skinner, M., Reid, W., El Bouhssini, M., 2005. Molecular characterization of *Beauveria bassiana* isolates obtained from overwintering sites of Sunn Pests (*Eurygaster* and *Aelia* species). Mycol. Res. 109, 294–296.

Burdon, J.J., Silk, J., 1997. Sources and pattern of diversity in plant pathogenic fungi. Phytopathology 87, 664–669.

Castrillo, L.A., Vendenberg, J.D., Wraight, S.P., 2003. Strain specific detection of introduced *Beauveria bassiana* in agricultural field by use of sequence-characterized amplified region markers. J. Invertebr. Pathol. 182, 75–83.

Coates, B.S., Hellmich, R.L., Lewis, L.C., 2002. *Beauveria bassiana* haplotype determination based on nuclear rDNA internal transcribed spacer PCR-RFLP. Mycol. Res. 106, 40–50.

Dalzoto, P.R., Glienke-Blanco, C., Kava-Cordeiro, V., Araujo, W.L., Azevedo, J.L., 2003. RAPD analysis of recombination processes in the entomopathogenic fungus *Beauveria bassiana*. Mycol. Res. 107, 1069–1074.

Ò'arriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Enkerli, J., Widmer, F., Gessler, C., Keller, S., 2001. Strain-specific microsatellite markers in the entomopathogenic fungus *Beauveria brongniartii*. Mycol. Res. 105, 1079–1087.

Estrada, M.E., Camacho, M.V., Benito, C., 2007. The molecular diversity of different isolates of *Beauveria bassiana* (Bals.) Vuill. as assessed using intermicrosatellites (ISSRs). Cell Mol. Biol. Lett. 12, 240–252.

Fernandes, E.K.K., Costa, G.L., Moraes, A.M.L., Zahner, V., Bittencourt, V.R.E.P., 2006. Study on morphology, pathogenicity, and genetic variability of *Beauveria bassiana* isolates obtained from *Boophilus microplus* tick. Parasitol. Res. 98, 324–332.

Fontecha, G., Tabanino, R., Perez-Borrero, B., Catalan, P., Aguilar, E., Gallego, F.J., Figueiras, A.M., Benito, C., 2011. Caracterización molecular de aislados Centroamericanos de *Beauveria bassiana* para el control de la broca del café. Rev. Cienc. Tecnol. 8, 39–61.

Gaitan, A., Valderrama, A.M., Saldarriaga, G., Velez, P., Bustillo, A., 2002. Genetic variability of *Beauveria bassiana* associated with the coffee berry borer *Hypothenemus hampei* and other insects. Mycol. Res. 106, 1307–1314.

Garrido-Jurado, I., Marquez, M., Ortiz-Urquiza, A., Santiago-Alvarez, C., Iturrriaga, A., Quesada-Moraga, E., Monte, E., Hermosa, R., 2011. Genetic analyses place most Spanish isolates of *Beauveria bassiana* in a molecular group with world-wide distribution. BMC Microbiol. 11, 84.

Glare, T.R., Inwood, A.J., 1998. Morphological and genetic characterization of *Beauveria* spp. from New Zealand. Mycol. Res. 102, 250–256.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. Nucl. Acid. Symp. Ser. 41, 95–98.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Lewis, L.C., Bruch, D.J., Gunarson, R.D., Binde, K.G., 2001. Assessment of plant pathogenicity of endophitic *Beauveria bassiana* in Bt transgenic and non-transgenic corn. Crop Sci. 41, 1395–1400.

Maurer, P., Couteaudier, Y., Girard, P.A., Bridge, P.D., Riba, G., 1997. Genetic diversity of *Beauveria bassiana* and relatedness to host insect range. Mycol. Res. 101, 159–164.

Ormond, E.L., Thomas, A.P.M., Pugh, P.J.A., Pell, J.K., Roy, H.E., 2010. A fungal pathogen in time and space: the population dynamics of *Beauveria bassiana* in a conifer forest. FEMS Microbiol. Ecol. 74, 146–154.

Rehner, S.A., 2005. Phylogenetics of the insect pathogenic genus *Beauveria*. In: Vega, F.E., Blackwell, M. (Eds.), Insect Fungal Associations. Ecology and Evolution. Oxford University, pp. 3–27.

Rehner, S.A., Buckley, E., 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97, 84–98.

Rehner, S.A., Minnis, A.M., Sung, G.H., Luangsa-Ard, J.J., Devotto, L., Humber, R.A., 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103, 1055–1073.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain – terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. 74, 5463–5467.

St Leger, R.J., Allee, L.L., May, B., Staples, R.C., Roberts, D.W., 1992. World-wide distribution of genetic variation among isolates of *Beauveria* spp. Mycol. Res. 96, 1007–1015.

Takatsuka, J., 2007. Characterization of *Beauveria bassiana* isolates from Japan using inter-simple-sequence-repeat-anchored polymerase chain reaction (ISSR-PCR) amplification. Appl. Entomol. Zool. 42, 563–571.

Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564–577.

Tamura, K., Peterson, D., Peterson, N., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Toledo, A.V., De Remes Lenicov, A.M.M., López Lastra, C.C., 2007. Pathogenicity of fungal isolates (Ascomycota: Hypocreales) against *Peregrinus maidis*, *Delphacodes kuscheli* (Hemiptera: Delphacidae) and *Dalbulus maidis* (Hemiptera: Cicadellidae), vectors of corn diseases. Mycopathologia 163, 225–232.

Toledo, A.V., De Remes Lenicov, A.M.M., López Lastra, C.C., 2008. Host range findings on *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales) in Argentina. Bol. Soc. Argent. Bot. 43, 211–220.

Vega, F.E., 2008. Insect pathology and fungal endophytes. J. Invertebr. Pathol. 98, 277–279.

Wang, C.S., Shah, F.A., Patel, N., Li, Z.Z., Butt, M.T., 2003. Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*. Environ. Microbiol. 5, 908–915.

Wang, S.B., Miao, X.X., Zhao, W.G., Huang, B., Fan, M.Z., Li, Z.Z., Huang, Y.P., 2005. Genetic diversity and population structure among strains of the entomopathogenic fungus, *Beauveria bassiana*, as revealed by inter-simple sequence repeats (ISSR). Mycol. Res. 109, 1364–1372.

Wang, J., Yang, L., Qiu, X., Liu, Y., Zhou, W., Wan, Y., 2013. Diversity analysis of *Beauveria bassiana* isolated from infected silkworm in southwest China based on molecular data and morphological features of colony. World J. Microb. Biotechnol. 29, 1263–1269.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols. A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.

White, J.F., Belanger, F., Meyer, W., Sullian, R.F., Bischoff, J.F., Lewis, E.A., 2002. Clavicipitalean, fungal epibionts and endophytes-development of symbiotic interactions with plants. Symbiosis 33, 201–213.

Zhang, Y.J., Zhang, S., Lui, X.Z., Wen, H.A., Wang, M., 2010. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Lett. Appl. Microbiol. 51, 114–118.