# RAPD, MICROSATELLITES MARKERS IN THE GENETIC DIVERSITY CHARACTERIZATION OF *Beauveria bassiana* (BALS.) VUILL. ISOLATES

# [RAPD, MICROSATÉLITES MARCADORES EN LA CARACTERIZACIÓN DE LA DIVERSIDAD GENÉTICA DE AISLADOS DE *Beauveria bassiana* (BALS.) VUILL.]

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## SUMMARY

*Beauveria bassiana* has been used for the control of agricultural pests. However, the genetic diversity of these fungi in the State of Sergipe, Brazil has been poorly studied. The objective of this study was to quantify the genetic diversity of *B. bassiana* isolates by means of RAPD markers, microsatellites, and rDNA-ITS regions, relating their origins and different geographic hosts. Nineteen primers were used in the RAPD analysis, and four primer pairs were used as microsatellite markers. The rDNA-ITS of 18S rDNA region were evaluated using primers ITS 1 and ITS 4. The RAPD analysis showed genetic similarities from 2% to 77%. Isolates 057.99 and 053.96 had the highest similarity (77%), followed by 064.99 and 053.96, with 73% similarity. With regard to microsatellites, isolates BC.05 and 032.91 showed the highest genetic similarity (82%), followed by 064.99 and 057.99 isolates, with 80% genetic similarity. The analysis of the rDNA-ITS region showed an intense fragment with 550pb for all isolates. PCR products were digested with *EcoRI* and *HaeIII*, but no restrictions were found for such products.

**Key words:** *Beauveria bassiana*; biological control; entomopathogenic fungi; molecular markers.

## RESUMEN

*Beauveria bassiana* se ha utilizado para el control de plagas agrícolas. Sin embargo, la diversidad genética de estos hongos en el Estado de Sergipe, Brasil ha sido poco estudiada. El objetivo de este estudio fue cuantificar la diversidad genética de *B. bassiana* aisladas por medio de marcadores RAPD, los microsatélites, y ADNr sus regiones, sobre sus orígenes y diferentes hosts geográfica. Diecinueve cebadores se utilizaron en el análisis RAPD, y cuatro pares de cebadores fueron utilizados como marcadores microsatélites. El ADNr 18S-ITS de la región de ADN recombinante se evaluó utilizando los cebadores ITS 1 e ITS 4. El análisis RAPD mostró similitudes genéticas del 2% al 77%. Aislados de 057,99 y 053,96 tuvieron la mayor similitud (77%), seguido por 064.99 y 053.96, con un 73% de similitud. Con respecto a los microsatélites, los aislamientos BC.05 y 032,91 mostraron la mayor similitud genética (82%), seguido de 064,99 y 057,99 aislados, con un 80% de similitud genética. El análisis de las ITS rDNA región mostró una banda intensa de todos los aislamientos. Los productos PCR fueron digeridos con *EcoRI* y *HaeIII*, pero no se encontraron restricciones para esos productos.

**Palabras clave**: *Beauveria bassiana*; control biológico; hongos entomopatógenos; marcadores moleculares.

## INTRODUCTION

*Beauveria* is a globally distributed genus of soil-borne entomopathogenic hyphomycetes of interest as a model system for the study of entomopathogenesis and the biological control of pest insects. Species recognition in *Beauveria* is difficult due to a lack of taxonomically informative morphology. This has impeded assessment of species diversity in this genus and investigation of their natural history (Rehner & Buckley, 2005). This globally distribution associated to difficulties for recognition species support the

objective of this current study in Sergipe State, due its important agriculture and the necessity to reduce the use of agrochemical products to control pests aiming the sustainability of agroecossistems.

The fungus *Beauveria bassiana* (Bals.) Vuill. has been used to control various insect species because of its great adaptation and persistence in the population of specific hosts and in the environment, producing effects, in the long run, on pest suppression (Alves, 1986).

Nowadays, many methods are available to analyze genetic diversity and to monitor entomopathogenic fungi. The methodologies can be separated based on the technique used, and vary with regard to their ability to detect differences between individuals, cost, ease of use, consistency, and reproducibility (Milach, 1998). Among these methods, RAPD and isozymes markers were used to discriminate 24 *B. bassiana* isolates collected from the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in North Carolina and West Virginia. The RAPD marker showed better resolution in the discrimination of isolates when compared with the isozymes, producing 141 bands for the 24 isolates and separating each as a unique class. Variation was detected not only within and between isolates from different regions, but also between isolates collected from the host insect (Castrilho & Brooks, 1998).

The detailed discrimination of the source of isolates, population structure, and genetic relations has turned the microsatellite technique into a frequently used method to differentiate entomopathogenic fungi. Rehner & Buckley (2003) described the isolation and characterization of eight microsatellite loci that successfully amplify some isolates representative of the phylogenetic diversity in the *B. bassiana* complex.

In addition, another genomic portion that can be of help in genetic diversity studies is ribosomal DNA (rDNA). The rDNA unit has components in its sequence which involve variations and can be used in systematic studies for different taxonomic levels PCR-restriction fragment length polymorphism (RFLP) assay of internal transcribed spacer (ITS) rDNA sequences (Fouly *et. al*, 1997).

Traditionally the identification of microorganisms has been done by assessing the phenotype by morphological characteristics; however, this can lead to controversy regarding the identification of several species, and are not safe to separate individuals into species. Traditional methods for identification of studies involving fungi require infection, morphology and culture media. These methods are usually time consuming and can be inconclusive and lead to misinterpretation. The use of molecular techniques in study of enthomopathogenic fungi has been employed. These tools have also been used in the analysis of genetic diversity and the study of intra-and interspecific relationships of different species, populations and individuals. Such studies have been performed by analysis of macromolecules through the use of molecular markers, which are based on the variation found in protein or in the sequence of nucleotides that compose the nucleic acids DNA and RNA, which has been used to facilitate understanding of the taxonomy, phylogeny and ecology of various fungi (Kao *et al*., 2002; Gauthier *et al*., 2007).

Therefore, the objective of this study was to evaluate the genetic variability among isolates of the fungus *B. bassiana*, collected from different hosts and regions in the State of Sergipe and other localities, by means of RAPD markers, microsatellites, and amplification of the rDNA ITS region.

## MATERIAL AND METHODS

### *Beauveria bassiana* isolates and culture conditions

We used 11 isolates of the entomopathogenic fungus *B. bassiana*, collected from different locations and naturally parasitized insect species in the field (Table 1).

The isolates were deposited in Embrapa *Tabuleiros Costeiros*' Biological Agents Bank and then transferred to liquid medium (40 mL) consisting of: sucrose - 10g $L^{-1}$, L-asparagine – 2g $L^{-1}$, yeast extract – 2g $L^{-1}$, $KH_2PO_4$ – 1g $L^{-1}$, $MgSO_4.7H_2O$ – 0.1g $L^{-1}$, $ZnSO_4.7H_2O$ – 0.44mg $L^{-1}$, $FeCl_3.6H_2O$ – 0.48mg $L^{-1}$, $MnCl_2.H_2O$ – 0.36mg $L^{-1}$ (Alfenas, 1998). The fungi were maintained in this medium at 25ºC±1ºC for 7 days, on a stirring table at 80 *g* in the dark. After that period, the mycelia were vacuum-filtered through a Büchner funnel, stored at -21ºC in freezer and then freeze-dried (Benchtop device, Virtis, NY, USA).

### DNA extraction

For genomic DNA extraction, 700 µL of a solution consisting of 549 µL sterile water, 300 µL 10% SDS, 100 µL 0.5M EDTA pH 8, and 50 µL 1M Tris-HCl pH 8, and 2-mercaptoethanol/sample were added to the mycelium macerated in liquid nitrogen, which was maintained at 65ºC for 60 min.

The mixture was centrifuged at 12,000 *g* for 10 min and the supernatant was collected. Six-hundred µL of a saturated phenol and chloroform mixture (1:1) were added, followed by centrifugation at the same rotation and for the same time. The supernatant was transferred to a microtube, and the same volume of chloroform and isoamyl alcohol (1:1) was added. The mixture was centrifuged and the supernatant was removed; 1/10 of

its volume of 3M sodium acetate and 2/3 of ice-cold isopropanol (-20ºC) were added.

After resting for 60 min at -20ºC, the mixture was centrifuged and the precipitate thus obtained was washed twice with 200 µL 70% ethanol. The precipitate was resuspended with 100 µL TE (Tris-EDTA buffer) containing RNAase (10 ng $\mu L^{-1}$) and incubated at 65ºC for 20 min. Two new precipitations and washes were performed, and the precipitate was resuspended with 50 µL TE. All amplification reactions were performed in a thermocycler (Biômetra, complete description).

**Amplification of RAPD markers**

The amplification reactions had a 25 µL volume [14.3 µL sterile nuclease-free water, 3 µL 10X buffer, 0.6 µL d-NTP´s (10mM), 0.9 µL $MgCl_2$ (50mM), 0.2 µL taq polymerase (2U), 3 µL primer, and 3 µL DNA]. Twenty arbitrary sequence decamer primers (IDT 1 to 10) were tested.

Amplification consisted of an initial temperature of 94°C/5 min followed by 45 amplification cycles at 94°C/1 min; 36°C/2 min; and 72°C/1 min.

The amplification products were separated on 0.8% agarose gel under electrophoresis in TBE buffer 0.5X (90mM Tris-borate, 1mM EDTA, pH 8.0) at 100V for 90 minutes, stained with ethidium bromide (0.5 µg mL) and visualized under UV light.

**Microsatellite markers in *Beauveria bassiana* diversity analysis**

The DNA extracted from *B. bassiana* isolates was submitted to PCR reactions using four pairs of microsatellite marker-based primers (Rehner & Buckley, 2003).

The amplification reactions were conducted in a 25µL volume [3µL buffer 10X, 0.9µL $MgCl_2$ (50mM), 0.6µL d-NTP's (10mM), 0.4µL taq polymerase (2U), 18.1µL ultrapure water, 0.5µL of each primer (sense and antisense at 0.2 µM), and 1 µL (30ng) DNA] in a thermocycler programmed for 94ºC/5 min, followed by 30 cycles at 94ºC/1 min, 50ºC/1 min, 72ºC for 2 min, and finally 72ºC/7 min. The products were separated in 1.5% agarose gel under electrophoresis in TBE buffer 1X, and then visualized under UV light.

**Amplification of rDNA ITS regions**

The primers used were synthesized by GIBCO (Life Technologies) (ITS1- TCC GTA GGT GAA CCT GCG G and ITS4- TCC TCC GCT TAT TGA TAT GC) (White *et al*., 1990), and suspended in sterile ultrapure water to a concentration of 200 µM. The working solutions were used at concentrations from 20 to 30 µM.

The reactions were conducted in 25 µL volumes [16.4 µL sterile ultrapure water; 2.5 µL PCR buffer 10x; 2.5 µL $MgCl_2$; 0.5 µL d-NTP's; 0.5 µL of each primer; and 0.1 µL Taq DNA polymerase per reaction, and 3 µL DNA]. The amplification program consisted of initial denaturation at 95°C/3 min; followed by 32 cycles at 94°C/1 min, 57°C/1 min, and 72°C/1 min; and one cycle at 72°C/3 min.

The PCR products were separate on a 1.5% agarose gel under electrophoresis at 1.5%/TBE 1X, stained with ethidium bromide, photographed using UV light source.

Table 1 - Monospore *Beauveria bassiana* (Bals.) Vuill. cultures from the Biological Agents Bank at Embrapa *Tabuleiros Costeiros*.

| Isolates | Isolation location | Host species/Family |
|---|---|---|
| BC.05 | Boquim/SE – C | *Cratosomus flavofasciatus*/Curculionidae |
| 027.94 | São Cristóvão/SE – C | *Rhinostomus barbirostris*/Curculionidae |
| 032.91 | Aracaju/SE – C | *Rhynchophorus palmarum*/Curculionidae |
| 052.96 | Betume1/SE – C | *Homalinotus coriaceus*/Curculionidae |
| 053.96 | Moju/PA – I | *Brassolis sophorae*/Nymphalidae |
| 057.99 | Betume2/SE – C | *Homalinotus coriaceus*/Curculionidae |
| 058.99 | Saquarema/RJ – I | *Homalinotus coriaceus*/Curculionidae |
| 059.99 | Malhador/SE – C | *Rhynchophorus palmarum*/Curculionidae |
| 062.00 | Neópolis/SE – C | *Coraliomela brunnea*/Chrysomelidae |
| 064.99 | Egypt – I | *Rhynchophorus ferruginus*/Curculionidae |
| 065.03 | Umbaúba/SE – C | *Rhinostomus barbirostris*/Curculionidae |

C – Collected; I – Introduced

**ITS-RFLP**

The ITS region product amplified by PCR was digested with *HaeIII* and *EcoRI* endonucleases (5µL), for 4 hours at 37°C. Electrophoresis was run from the digested product at 80 V and 80mA for 3 hours, in 1.5% agarose gel, using TBE buffer 1X, and then stained with ethidium bromide (0.5 µg $mL^{-1}$) and visualized under UV light.

**Data analysis**

When evaluating the gels, the presence (1) or absence (0) of bands were used to construct matrices. The genetic similarity estimates (Sg*ij*) between each pair of genotypes were obtained using Jaccard's coefficient by means of the expression: Sg*ij* = a/a+b+c.

The variables for the expressions were obtained according to the following scheme:

| | | Isolate i | |
|---|---|---|---|
| | | 1 | 0 |
| Isolate j | 1 | a (1, 1) | b (1, 0) |
| | 0 | c (0, 1) | d ( 0, 0) |

Similarities were obtained using the statistical package NTSYS-pc version 2.1, while clusters were obtained by the UPGMA method (Rohlf, 2000). The errors associated with the similarities were estimated according to Skroch *et al*. (1992), by the following expressions:

V= ns (1-s) (n-1), estimated standard error $(V/n)^{1/2}$

where:
V= variance for the genetic similarity between each pair of isolates;
s= genetic similarity between each pair of isolates;
n= total number of bands used to estimate similarities.

## RESULTS AND DISCUSSION

In the RAPD analysis, 38 polymorphic bands and 14 monomorphic bands were obtained, some unique for certain isolates. These were used to calculate genetic similarity values, which ranged from 2% to 77%.

The isolates showed a mean similarity of 38.6%, forming distinct groups on the dendrogram, even among those isolates that came from common hosts; isolate 65.03 was the most divergent as compared to the others (Figure 1).

Based on the genetic similarities between the analyzed genotypes (Table 2), isolates 57.99 (Betume2-SE) and 53.96 (Moju-PA) showed the highest similarity (77%), followed by 64.99 (Egypt) and 53.96 (Moju-PA), with 73% similarity.

Ferreira *et al*., (2001) conducted bioassays comparing the pathogenicity of isolates 57.99 and 53.96 on *B. sophorae*, the coconut leaf caterpillar. The virulence shown during control of this insect was similar, around 92.5%, corroborating the RAPD test results in which the highest genetic similarity value was observed (77%). These isolates also showed potential for the biological control of other coconut pests, such as: *H. coriaceus* - black coconut bunch weevil (87%), *R. palmarum* - American palm weevil (91.5%), and *R. barbirostris* - bottle brush weevil (95.3%).

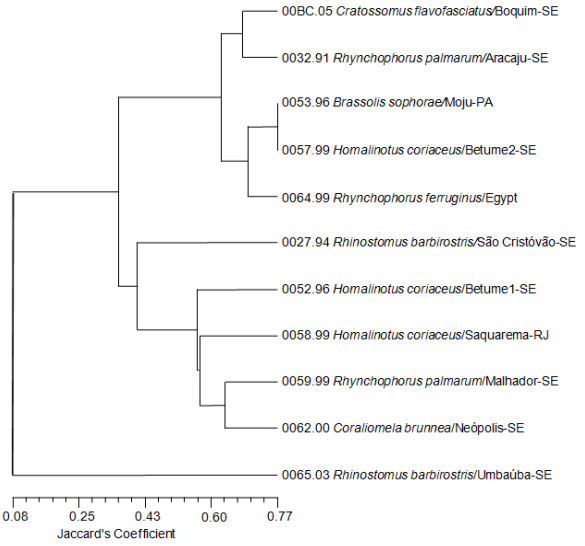


Figure 1. Dendrogram of similarities between 11 *Beauveria bassiana* isolates for RAPD analysis, based on Jaccard's similarity coefficient (UPGMA method).

Isolates 57.99 and 58.99, obtained from the same host (*H. coriaceus*), showed 30% genetic similarity. A similar result was observed between isolate pairs 32.91 and 58.99, and 32.91 and 59.99, obtained from *R. palmarum*, with a mean genetic similarity of 36.5% (Table 3). Among these isolates, 32.91 has caused up to 100% mortality in *R. palmarum* adults in laboratory tests, followed by isolates 59.99, 57.99, and 58.99 (Ferreira *et al*, 2001).

The isolates from Sergipe with the highest genetic similarity were BC.05 (Boquim-SE) and 32.91 (Aracaju-SE) (68%), which were obtained from insect pests on different crops (*Citrus* and *Cocus nucifera*), but belonged to the same family (Curculionidae). Conversely, those with the lowest similarity were 65.03 (Umbaúba-SE) and 59.99 (Malhador-SE), both isolated from a coconut pest.

Isolate BC.05 was collected in 2005. So far, no evaluation tests on its pathogenicity have been carried out. Nevertheless, due to its close genetic proximity with isolate 32.91, it has a potential for the control of *R. palmarum*, since it was obtained from an insect - *C. flavofasciatus* - in the same family. Similarly, it can be suggested that isolate 32.91 has a potential to control *C. flavofasciatus*.

The isolates with the smallest similarity values were 65.03 and 59.99 (2%). These isolates were obtained from distinct edaphic-climatic regions: one was from Umbaúba (latitude 11º23'00" south/longitude 37º39'28" west), at an elevation of 130 meters, and the other was from Malhador (latitude 10º39'28" south/longitude 37º18'17" west), at an elevation of 251 meters, with variable rainfall regimes and mean temperatures in those regions (SEPLANTEC, 2006). These factors may act as selection pressure for the occurrence of mutations between isolates.

The microsatellite analysis for *B. bassiana* generated 41 bands, all of which were polymorphic; the electrophoretic migration distances between amplification products were different from the populations described by McGuire *et al*. (2005), possibly due to their various origins and hosts of the isolates used in the analysis.

A total of 20 amplified bands were observed for primer sequence Ba01; the highest polymorphism was observed for primer Ba05, with only two amplified bands. The length of the amplified fragments varied from 100 to 2,000 bp.

Rehner and Buckley (2003) evaluated a wide geographic population of *B. bassiana* and observed that the size of the fragments amplified by the Ba05 sequence varied from 110 to 175 bp.

The highest genetic similarity values observed among the isolates analyzed (Table 3) were 82% for pairs BC.05 (Boquim - SE) and 32.91 (Aracaju - SE), and 80% for 64.99 (Egypt) and 57.99 (Betume2-SE). For the first pair, the microsatellites confirmed the RAPD analysis results, where a higher genetic similarity value was observed for these isolates, both from Sergipe (Figure 2).

Table 2. Genetic similarities (%) (located below the diagonal line) and standard error associated with similarity (above the diagonal line) between *Beauveria bassiana* isolates based on Jaccard's coefficient for RAPD markers.

| Isolates | BC05 | 27.94 | 32.91 | 52.96 | 53.96 | 57.99 | 58.99 | 59.99 | 62.00 | 64.99 | 65.03 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BC05 | | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.001 |
| 27.94 | 44 | | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.002 | 0.001 |
| 32.91 | 68 | 37 | | 0.003 | 0.003 | 0.002 | 0.003 | 0.003 | 0.003 | 0.003 | 0.001 |
| 52.96 | 43 | 47 | 46 | | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.001 |
| 53.96 | 55 | 33 | 68 | 44 | | 0.002 | 0.003 | 0.003 | 0.003 | 0.002 | 0.001 |
| 57.99 | 64 | 34 | 69 | 37 | 77 | | 0.002 | 0.003 | 0.003 | 0.003 | 0.001 |
| 58.99 | 33 | 43 | 35 | 55 | 35 | 30 | | 0.003 | 0.003 | 0.002 | 0.0004 |
| 59.99 | 32 | 35 | 38 | 56 | 38 | 32 | 58 | | 0.003 | 0.003 | 0.0002 |
| 62.00 | 36 | 37 | 36 | 57 | 36 | 32 | 55 | 64 | | 0.002 | 0.0006 |
| 64.99 | 51 | 30 | 67 | 35 | 73 | 66 | 29 | 32 | 30 | | 0.001 |
| 65.03 | 11 | 9 | 8 | 9 | 10 | 10 | 4 | 2 | 5 | 8 | |

Table 3. Genetic similarities (%) (below the diagonal line) and standard error associated with similarity (above the diagonal line) between *Beauveria bassiana* isolates using microsatellites, based on Jaccard's coefficient.

| Isolates | BC05 | 27.94 | 32.91 | 52.96 | 53.96 | 57.99 | 58.99 | 59.99 | 62.00 | 64.99 | 65.03 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BC05 | | 0.003 | 0.002 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.000 |
| 27.94 | 54 | | 0.003 | 0.003 | 0.002 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.001 |
| 32.91 | 82 | 48 | | 0.003 | 0.003 | 0.002 | 0.002 | 0.003 | 0.003 | 0.002 | 0.000 |
| 52.96 | 59 | 59 | 45 | | 0.002 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.0004 |
| 53.96 | 32 | 26 | 37 | 20 | | 0.003 | 0.001 | 0.002 | 0.002 | 0.003 | 0.000 |
| 57.99 | 64 | 44 | 74 | 42 | 63 | | 0.002 | 0.003 | 0.003 | 0.002 | 0.000 |
| 58.99 | 37 | 46 | 31 | 50 | 12 | 26 | | 0.003 | 0.003 | 0.003 | 0.001 |
| 59.99 | 56 | 56 | 50 | 61 | 19 | 41 | 53 | | 0.003 | 0.003 | 0.001 |
| 62.00 | 50 | 50 | 43 | 62 | 17 | 35 | 54 | 67 | | 0.003 | 0.0004 |
| 64.99 | 62 | 36 | 72 | 40 | 45 | 80 | 32 | 44 | 37 | | 0.000 |
| 65.03 | 0 | 9 | 0 | 4 | 0 | 0 | 14 | 8 | 4 | 0 | |

Enkerli *et al.* (2004) used microsatellite markers for the genetic characterization of *B. brongniartii* isolates applied during 14 years for the biological control of *Melolontha melolontha* (Coleoptera: Scarabaeidae), and demonstrated that after that period some isolates persisted in the environment and new populations were derived from them, coexisting in the same habitat. This fact has probably contributed for other factors to act as selection pressure over this species.

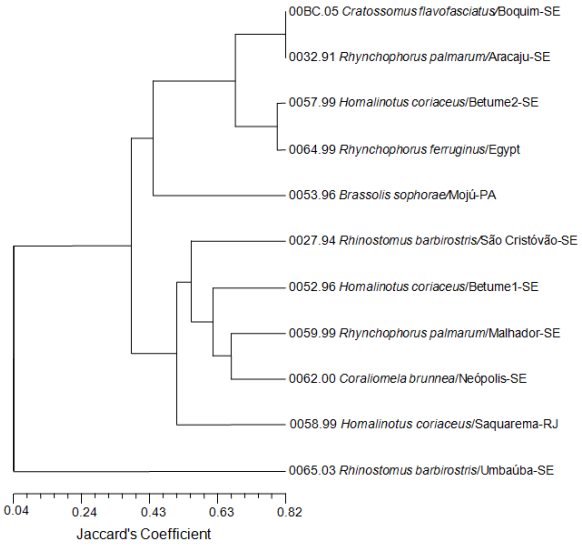


Figure 2. Dendrogram of similarities between 11 *Beauveria bassiana* isolates for microsatellites, based on Jaccard's similarity coefficient (UPGMA method).

A similar result as obtained in the RAPD analysis was observed in the microsatellite analysis for isolate 65.03, with no genetic similarity with isolates BC.05, 32.91, 53.96, 57.99, and 64.99. This fact validates the information obtained with multiple molecular markers in studies on populations of entomopathogenic fungi.

Figure 3 confronts the genetic diversity data for *Beauveria* utilizing isolates using RAPD molecular markers and microsatellites in the consensus analysis. It can be seen that two great groups were formed, with approximately 38% genetic divergence. One group includes isolates BC.05, 32.91, 53.96, 57.99, and 64.99, while the other group includes isolates 27.94, 52.96, 58.99, 59.99, and 62.00. Isolate 65.03 formed a third independent branch, with 80% divergence, when compared with the others.

In order to elucidate the taxonomic position of isolate 65.03 in relation to the others with regard to the genetic proximities as evaluated by RAPD and microsatellites, a rDNA ITS region analysis was conducted, since this region is conserved within a species but is variable between species. A band of approximately 550 bp was observed for all isolates, in addition to the occurrence of a few unspecific bands with poor resolution.

Since no variations were observed in the amplification of the ITS regions, the amplification products were restricted for the various isolates. After restriction and visualization of the products, it was verified that no results occurred that would allow their differentiation.

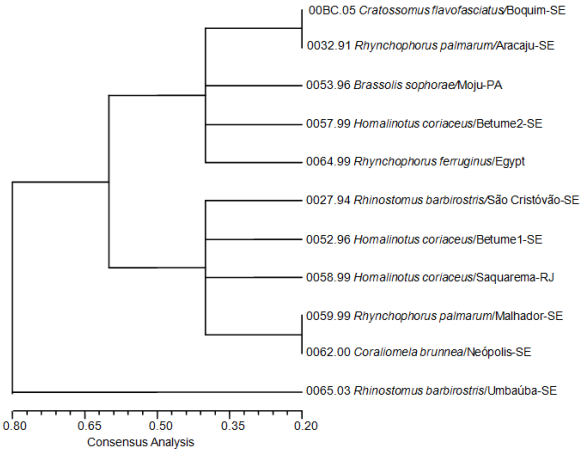


Figure 3. Dendrogram of consensus cluster analysis between 11 *Beauveria bassiana* isolates for RAPD and microsatellites, based on Jaccard's coefficient (Strict method).

On the other hand in a study of *Metarhizium anisopliae var. anisopliae* isolates the ITS regions resulted in a single PCR-amplification product for all isolates, while digestion of the ITS1 and ITS2 PCR-amplification products with several restriction enzymes detected several molecular differences. The conservative nature of our ITS fingerprinting analysis meant that it was very difficult to discriminate between isolates because many of the isolates analyzed shared the same genotype (Becerra Velásquez *et al*., 2007).

The formation of two great groups was observed in the dendrogram. Group 1 consisted of isolates BC.05, 32.91, 64.99, 27.94, 58.99, 62.00, and 65.03, while group 2 consisted of isolates 52.96, 53.96, 57.99, and 59.99.

In evaluating isolates from the same genus, *Rhynchophorus*, isolates 32.91 (*R. palmarum*) and 64.99 (*R. ferruginus*) showed 50% genetic similarity. However, considering the intraspecific values for this genus, a low similarity (17%) was observed in isolates 32.91 and 59.99 (*R. palmarum*). A similar fact was found in the species *H. coriaceus*, for which a low

intraspecific genetic similarity was observed in pair 52.96 and 58.99 (25%) (Figure 3).

The populations of isolates from the State of Sergipe showed genetic diversity. Knowledge about the distribution and magnitude of intra- and interpopulation genetic variation is of great importance to understand the fungus biology, which could be used to make inferences about the potential impact of different ecological factors that influence the evolution process of the species (Wang, *et al.*, 2003).

Wang *et al.* (2003) worked with microsatellite markers and observed that the genetic relation between *B. bassiana* isolates was more associated with geographic location than with insect host species, suggesting that the location factor constitutes selection pressure. In this work, no direct relation was observed between similarity data, geographic location, and host, as previously suggested.

Wang *et al*. (2004) used polymerase chain reaction-restriction fragment length polymorphism PCR-RFLP of the *pr1* gene, microsatellite markers and 28S rDNA haplotyping detecting the presence or absence of group I introns in the population study of the entomopathogenic fungus, *Beauveria bassiana*. The findings showed that the average genetic diversity index of geographical populations was significantly smaller than that of populations derived from insect host orders, indicating that the genetic relatedness of *B. bassiana* strains was highly associated with geographical locality rather than insect host species.

In the Sergipe conditions, successive applications of *B. bassiana* have been used for the biological control of coconut and citrus pests. Under favorable conditions, the fungus persists in the environment through the generations of insects present, and may undergo the action of different management practices and environmental conditions which, throughout the years, would imply selection pressures that might generate new genetic types.

Thirty-eight strains of the entomopathogenic *B. bassiana*, isolated from diverse species of Lepidoptera (Pyralidae) or Coleoptera (Curculionidae, Chrysomelidae, and Scolytidae) from various geographical sites, were examined by RFLP and RAPD analysis, showing clustering genus host specifity (Mauer *et al*., 1997), which differs from those results reported in this study.

Using *Paecilomyces farinosus* genus a study was determined by comparison of the products of polymerase chain reaction amplification of anonymous regions of genomic DNA with single arbitrary sequence oligonucleotide primers (RAPD analysis). Isolates were collected from seven insect species in eastern Canada and they differed greatly in cultural and morphological phenotype. All *P. farinosus* isolates were clearly distinguished from three other entomopathogenic fungi, including *P. fumosoroseus*. However, RAPD banding patterns did not, correlate with ecological backgrounds or morphological phenotypes of *P. farinosus* isolates. These observations support the conclusion that *P. farinosus* from eastern Canada is not composed of strains which can be separated on the basis of the ecological or morphological criteria selected (Chew *et al*., 1998).

New perspectives do come up and may lead to a necessity for researches focused on the specific identification of the most divergent isolates of this pathogen for various crops. Such identification may suggest a new taxonomic position, and the identification of strains of these *B. bassiana* isolates which, after characterization, would allow the identification of markers associated with pathogenicity, contributing toward the selection of more virulent types in a more rapid and precise manner, associated with the evaluation via inoculation and later determination of pest mortality.

## CONCLUSIONS

*Beauveria bassiana* isolates showed a mean similarity of 38.6% for RAPD analysis. The isolates 57.99 (Betume2-SE) and 53.96 (Moju-PA) showed the highest similarity (77%) by RAPD analysis. By microsatellite analysis, the isolates BC.05 (Boquim-SE) and 32.91 (Aracaju-SE) showed the highest genetic similarity (82%). The ITS products, obtained for the different isolates of *Beauveria bassiana*, confirmed higher homology of the sequences for the analyzed isolates.

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