Genetic diversity of *Beauveria bassiana* in semi natural and agricultural habitats and its biocontrol potential against cowpea aphid, *Aphis craccivora* Koch

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Received: 24 January 2019 / Accepted: 7 June 2019 / Published online: 19 June 2019
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Abstract

Information on the biology and ecology of *Beauveria bassiana* in different habitats could provide essential knowledge in their development as biocontrol agents of insect pests. In this study, phylogenetic and genotypic information was used to evaluate the genetic diversity of *B. bassiana* within semi natural and agricultural habitats in Karnataka State of South India and assessed their extracellular chitinase activity and pathogenicity against cowpea aphid, *Aphis craccivora*. Multilocus phylogeny and microsatellite genotyping of *B. bassiana* conjointly resolved three phylogenetic species, Bb_1, Bb_2, and Bb_3, in semi natural and agricultural habitats. None of the three phylogenetic species of *B. bassiana* were associated with crop plants in agroecosystem or insect hosts in semi natural habitat. All the three phylogenetic species were detected with four genotypes each. All isolates of *B. bassiana* were pathogenic to *A. craccivora* in greenhouse bioassays. Isolate GKVK 01_13 caused a significantly high mortality of aphids and detected with an increased level of chitinase activity. The study results suggest that application of indigenous virulent strain of *B. bassiana* could provide effective control of native insect pest *A. craccivora*.

Keywords Genetic diversity · *Beauveria bassiana* · Biocontrol · Chitinase activity · *Aphis craccivora*

Introduction

Beauveria bassiana (Bals.) Vuillemin (Ascomycota: Hypocreales) is a cosmopolitan anamorphic hyphomycete known for their role as insect pathogens [1, 2]. They cause white muscardine disease in insects. *B. bassiana* was found and isolated from soil [3] and insects belonged to different orders [4]. They infect a wide range of insect pests, and this ability has been explored for the development of commercial products of the fungus for the biocontrol of insect pests [5]. Studies on *B. bassiana* showed that they are nonmonophyletic and contains distantly related morphologically cryptic species

[6, 7]. Species identification and genotype recognition of *B. bassiana* can be achieved with the aid of molecular phylogenetic markers and microsatellite markers [6, 8, 9].

Knowledge on biology and ecology of *B. bassiana* in various habitats could provide essential information for their development as biocontrol agents [10]. Information on genetic diversity and genotyping is critical to investigate strain dependent virulence and host-pathogen interactions of strains which will be developed as microbial insecticides [11]. Different strains of *B. bassiana* exhibit considerable variation in virulence and host range. Research on population genetic structure of *B. bassiana* showed that specific genotypes were adapted to particular habitats not to insect host [9, 10, 12]. Multilocus phylogeny of *B. bassiana* revealed the occurrence of multispecies sympatry within single insect host [6, 13]. A limited number of studies are available on the regional diversity and distribution of *B. bassiana* in various habitats of India [14, 15].

Cowpea, *Vigna unguiculata* (L.) Walp., is an economically important vegetable and grain legume that represents a major protein component of the diet in tropics. Cowpea aphid, *Aphis craccivora* (Koch), is a major sucking insect pest of legumes (Fabaceae) that also attacks plants of at least 19 other families [16]. Incidence of aphid in cowpea has been observed in

Responsible Editor: Ieda Carvalho Mendes.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s42770-019-00102-5>) contains supplementary material, which is available to authorized users.

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almost all states of India. Aphid causes 20–40% yield loss of cowpea in India and they act as a vector in the transmission of cowpea mosaic disease leading to 80–100% loss [17]. Development of biocontrol alternatives is essential to replace the current use of conventional synthetic insecticides to manage them. Deployment of fungal entomopathogens in the biocontrol of sucking insect pest is appropriate as the pathogens infect by direct contact and do not require ingestion for infection.

During the process of infection, *B. bassiana* secrete enzymes such as chitinase, protease, esterase, and lipase to degrade cuticle components [18–21] and these enzymes are crucial in the mechanism of antagonistic activity of biocontrol agents. In spite of the vast interest in developing commercial formulations of *B. bassiana* against various insect pests of agroecosystem, progress towards to understand their genetic diversity, strain-associated virulence, and host and habitat adaptations are limited in India. Though *B. bassiana* are known for its natural epizootics in diverse insects of different habitats, knowledge on the ecology and biology of this pathogen could improve its prospects in the development of fungal insecticides. This study is aimed to determine the phylogenetic and genotypic diversity of *B. bassiana* within cultivated agroecosystem and semi natural habitat in Karnataka State of South India and to assess their extracellular chitinase activity and pathogenicity against cowpea aphid, *A. craccivora*, to determine the biocontrol potential.

Materials and methods

Collection, isolation, and culture of isolates

Isolates of *B. bassiana* were collected from insect hosts of semi natural habitat and cultivated agricultural soil in the south part of Karnataka State of India in late 2012 and early 2013 (Table S1). Three plots (50 m²) were randomly laid out in semi natural habitat (<0.30 ha) and insect cadavers were collected in every month for a period of 6 months. Ten crop plants such as areca nut (*Areca catechu*), cauliflower (*Brassica oleracea*), chili (*Capsicum annuum*), cardamom (*Ellattaria Cardamomum*), finger millet (*Eleusine coracana*), field bean (*Vicia faba*), french bean (*Phaseolus vulgaris*), pigeon pea (*Cajanus cajan*), ridge gourd (*Luffa acutangula*), and sugarcane (*Saccharum officinarum*) were selected for the collection of soil samples. Soil containing rhizosphere of crop plant was collected at a depth of 10 cm using a spade. Crop field (<0.20 ha) of younger plantings were selected for the collection of soil samples. Three soil samples of 500 g were randomly collected in a plastic bag (15 × 25 cm) from each crop field. These three sub samples were mixed thoroughly in a plastic bag (20 × 30 cm) to make pooled sample

of that field. Soil samples were brought to the laboratory and stored at 4 °C until use (1–2 days).

Isolates of *B. bassiana* from soil were obtained by “*Galleria* bait method” [22]. Soil samples were sieved to remove large particles. Ten 3rd instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae) were released into a ventilated plastic box (10 × 15 cm) containing 200 g of soil and incubated in dark at 18 ± 2 °C. Dead larvae were removed every 24 h interval until complete death and transferred to a moist filter paper (Whatman #1). Larval cadavers were incubated at 25 ± 1 °C for growth of the fungi. Fungus was identified using light microscope (Olympus BX51) after sporulation. Of the 10 crops surveyed, 2 crops, sugarcane and finger millet, yielded *Beauveria*. Infected insects from semi natural habitat and soils of agroecosystem yielded 11 and 4 isolates of *B. bassiana* respectively (Table S1). *B. bassiana* was prepared as single conidial isolates by randomly selecting a single colony forming unit (CFU) on Sabouraud dextrose yeast extract agar (SDYA). Isolates of *B. bassiana* were cryopreserved in the microbial collection at Department of Biotechnology, University of Agricultural Sciences, GKVK, Bangalore (Isolate collection code, GKVK).

DNA extraction, PCR, sequencing, and data preparation

Isolates of *B. bassiana* were individually grown in 50 ml sterile Sabouraud’s dextrose yeast extract broth (SDYB) at 120 rpm at 25 °C for 4 days. Mycelia were washed in distilled water and 50 mg of the mycelia were crushed with liquid nitrogen. Fungal DNA was extracted from the mycelia using CTAB method [23] and pellets of DNA were suspended in 1X TE buffer (pH 8.0). Three nuclear loci, Bloc an intergenic region, 3’ Translation Elongation Factor-1 alpha (3’ TEF1- α), and ribosomal internal transcribed spacer (ITS rDNA), were amplified according to Rehner et al. [6], Rehner et al. [7], and White et al. [24], respectively. Locus Bloc, amplified with primers B5.1F (5’-CGAC CCGCCAACTACTTTGA) and B3.1R (5’-GTCT TCCAGTACCACTACGCC), is suitable to identify the phylogenetic species within the genus *Beauveria* [6]. Locus TEF1- α , amplified with primers 983F (5’-GCYCCYGGHCAYCGTGAYTTYAT) and 2218R (5’-ATGACACCRACRGCRACRGTYTG), is used to separate the phylogenetic clades within *B. bassiana* [7]. Locus ITS rDNA was amplified with primers ITS5F (5’-GGAA GTAAAAGTCGTAACAAGG) and ITS4R (5’-TCCT CCGCTTATTGATATGC) [24]. Partial bidirectional nucleotide sequences were determined commercially for 15 isolates of *B. bassiana* for three loci. Alignment and editing of *B. bassiana* sequences with published sequences were carried out in Mega 5.05 [25] using default parameters.

Phylogenetic analysis

Molecular phylogenies of the sequences Bloc, TEF1- α , and ITS rDNA were used to infer phylogenetic diversity of *B. bassiana* in the Karnataka State. Phylogenetic tree was prepared using maximum likelihood (ML) and ML bootstrap analyses in raxmlGUI 1.3 [26]. Initially, sequences of Bloc, TEF1- α , and ITS loci were analyzed individually and the following analyses were carried on the combined sequence dataset. All ML searches for the shortest trees used addition of 1000 random pseudo sequence replicates with tree bisection and reconnection branch swapping (TBR). Nonparametric rapid bootstrapping method was conducted to assess the support of clades. ML tree on partitioned dataset was generated using GTR + G model of nucleotide substitution.

Initially, phylogenetic tree was constructed for 15 experimental isolates, one published isolate of *B. bassiana* and *B. brongniartii*, and one experimental isolate of *Isaria fumosorosea*, which produced three major clades. Therefore, phylogenetic tree for six representative experimental isolates, one published isolate of *B. bassiana* [6] and *B. brongniartii* [8], and one experimental isolate of *I. fumosorosea*, was reconstructed. Bloc, TEF, and ITS sequence matrix of 1493, 803, and 548 bp respectively were aligned to form sequence matrix of 2844 bp. Phylogenetic analyses were rooted to *I. fumosorosea*. GenBank accession numbers of the six representative experimental isolates of *B. bassiana* for Bloc, TEF, and ITS are GKVK 01_01 isolate KJ855045, KM091826, and KM114966; GKVK 01_54 isolate KJ855058, KM091839, and KM114979; GKVK 01_11 isolate KJ855050, KM091831, and KM114971; GKVK 01_14 isolate KJ855053, KM091834, and KM114974; GKVK 01_02 isolate KJ855046, KM091827, and KM114967, and GKVK 01_03 isolate KJ855047, KM091828, and KM114968, respectively. GenBank accession numbers of *I. fumosorosea* GKVK 01_32 isolate for TEF and ITS are KM091841 and KM114981, respectively. Phylogenetic tree was visualized in FigTree v.1.4 and the figure was sharpened in CorelDRAW X5.

Microsatellite genotyping

Different genotypes within the 15 isolates of *B. bassiana* were studied with 23 SSR markers; Ba01, Ba02, Ba03, Ba05, Ba06, Ba08, Ba12, Ba13, Ba14, Ba15, Ba16, Ba17, Ba18, Ba20, Ba21, Ba22, Ba23, Ba24, Ba25, Ba26, Ba27, Ba28, and Ba29 [9, 27]. PCR was performed in 20 μ l reaction mixture containing 20 ng genomic DNA, 25 mM MgCl₂, 2.5 mM of each dNTP, 10 pmol of each primer (Eurofin genomics, Bangalore), and 3 U of Taq DNA polymerase (GeNei, Bangalore) in 1X PCR buffer. PCR amplification parameters of the loci included preliminary denaturation at 95 °C for

4 min, followed by 35 cycles at 95 °C for 30 s, 56 °C for 45 s, 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR amplicons were resolved on 3% Metaphor agarose gel containing ethidium bromide (0.5 μ g/ml) corresponding to 50 bp molecular weight marker (Genei, Bangalore) using 1X TAE buffer. Reproducible alleles were visually scored for the presence (1) or absence (0) of band using gel documentation system (Bio-Rad, Singapore). Population statistics of the microsatellite marker data were estimated in Popgene v. 1.32 [28] and the genotypes were identified using Jaccard's similarity coefficient in NTSYSpc v. 2.02 [29]. ML analysis on untransformed microsatellite allelic data was performed to resolve the interspecific differentiation across loci in raxmlGUI 1.3 using BINGAMMAI model [26].

Bioassay of *B. bassiana* against cowpea aphid

Cowpea aphid, *A. craccivora*, was maintained on cowpea grown in net house. Initially, pathogenicity of 15 isolates of *B. bassiana* was tested against adults of the aphid using a single concentration of 1×10^6 conidia/ml. Isolates for dose-response bioassay were selected based on their bioefficacy and sporulation on the media. Bioassays of the three highly pathogenic and one less pathogenic isolates of *B. bassiana* were conducted against adults of *A. craccivora*. Isolates of *B. bassiana* were mass cultured on SDYA at 25 ± 1 °C, $80 \pm 2\%$ RH, and 12:12 (L:D) photoperiod. Conidial suspension of the isolates was prepared from 14-day-old cultures using sterile distilled water containing wetting agent Tween 20 (0.1%). Improved Neubauer hemocytometer was used to count the conidia and four concentrations, 4×10^5 , 6×10^5 , 8×10^5 , and 1×10^6 conidia/ml, were prepared from the stock and used in bioassays.

Ten healthy adults of *A. craccivora* were released to 2-week-old cowpea seedling grown in paper cup placed in greenhouse. A standard atomizer, which expelled approximately 100 μ l of the inoculum on one squeeze, was used to spray 1 ml of the suspension on each seedling. Seedlings were covered with perforated polythene bag. Mortality of the aphids was counted at 24 h interval until complete death. Three replicates with 10 aphids were maintained for each concentration. Aphids sprayed with sterile water served as control. Treated aphids were maintained at 25 ± 2 °C, $80 \pm 2\%$ RH, and 12:12 (L:D) photoperiod. Dead aphids were collected and incubated for 3–4 days for sporulation, and randomly selected cadavers were inoculated on media. Presence of the test fungus was confirmed under light microscope after growth. One-way analysis of variance (ANOVA) was conducted on angular transformed percentage mortality values and significance in mean per cent mortality between concentrations was tested on transformed values using Duncan's multiple range test (DMRT) in SPSS v. 17 [30].

Estimation of chitinase activity

Four isolates of *B. bassiana* selected for bioassays were grown in chitin media for 12 days. Chitinase activity of the isolates in culture supernatant was estimated using acid-swollen chitin as the substrate. To prepare acid-swollen chitin, 10 g of chitin (purified powder from crab shells, Himedia, Mumbai) was suspended in 100 ml of HCl (35%, w/v) and the mixture was stirred at 5 min interval for 1 h at room temperature in a fume hood. The resulting acid-swollen chitin was repeatedly washed with chilled distilled water followed by 1% NaHCO₃ (w/v) wash and further dialyzed against cold distilled water. A reaction mixture containing 1 ml of culture supernatant, 1 ml of 1 M sodium acetate buffer (pH 4.7), and 1 ml of colloidal chitin (0.1 g) was incubated at 60 °C for 1 h followed by centrifugation at 12,000 rpm for 5 min at 6 °C. Following centrifugation, an aliquot of 0.75 ml of supernatant, 0.25 ml of 1% dinitrosalicylic acid (DNS) in 0.7 M NaOH, and 0.1 ml of 10 M NaOH were mixed in 1.5 ml Eppendorf tube and heated at 100 °C for 5 min. After cooling the reaction mixture to room temperature, absorbance was measured at 582 nm [31]. A calibration curve of N-acetyl-D-glucosamine (GlcNAc) as a standard was used to determine the concentration of reducing saccharide. One unit of chitinase enzyme activity was defined as the amount of enzyme that catalyzed the release of sugars equivalent to 1 μmol of GlcNAc per min. One-way ANOVA was performed to find the variation in chitinase activity between isolates of *B. bassiana* and the significant difference was tested using DMRT in SPSS v. 17 [30].

Results

Phylogenetic diversity

Maximum likelihood analysis of the combined Bloc, TEF, and ITS sequence data of *B. bassiana* isolates produced a

phylogenetic tree with three well-supported terminal clades (Fig. 1). The numbers of isolates for each of the three *B. bassiana* phylogenetic species are listed with the clade identifiers. Distributions of the isolates of *B. bassiana* within the semi natural and agricultural habitats and its phylogenetic position are listed in Table S1. Comparison of Bloc, TEF, and ITS sequences with published sequences placed all isolates of *Beauveria* within single species, *B. bassiana*. Soil-derived isolates from agroecosystem ($n = 4$) were placed in two terminal clades with strong bootstrap support. Insect-derived isolates from semi natural habitat ($n = 11$) were placed in all the three clades. Cow bug, *Otinotus oneratus* (Walker), derived isolates of *B. bassiana* were distributed in all the three clades and thus showed multispecies sympatry.

The three phylogenetic clades of *B. bassiana* were considered distinct phylogenetic species and represented them as such. Each of the phylogenetic species of *B. bassiana* was designated as “Bb_X,” where “Bb” refers to *B. bassiana* and “X” to their clade number. It is evident that the most diverse phylogenetic species of *B. bassiana*, Bb_1, was isolated from soil of sugarcane and different insect hosts of semi natural habitat. All the three clades, Bb_1, Bb_2, and Bb_3, represents newly discovered phylogenetic species of *B. bassiana* isolated from soils of agricultural crops and insect hosts of semi natural habitat. In addition, 11 isolates of *B. bassiana* can be considered to be true fungal entomopathogens as they were obtained as infections in different insects of semi natural habitat.

Microsatellite genotypes

Twenty-three microsatellite markers were analyzed in the 15 isolates of *B. bassiana* collected from semi natural habitat and agroecosystem of Karnataka State. Twenty-two markers revealed typical microsatellite bands on 3% Metaphor agarose gel. Marker, Ba14, was failed to produce amplicons. Five markers, Ba01, Ba13, Ba16, Ba21, and Ba24, were found to be

Fig. 1 Phylogeny of *B. bassiana* inferred from maximum likelihood analysis of the combined Bloc, TEF, and ITS sequence data. Bootstrap values $\geq 70\%$ are listed above relevant internodes. The numbers of isolates for each of the phylogenetic species are listed with clade

monomorphic and 17 markers were polymorphic (Table S2). A total of 55 observed number of alleles was detected for the 22 markers in all isolates. The number of alleles per marker ranged from 1 to 6 in the collection. Nei's gene diversity (h) ranged from 0.12 to 0.76 with an average of 0.41 ± 0.06 in the collection. Shannon's Information index (I) ranged from 0.24 to 1.59 with an average of 0.71 ± 0.11 in the collection. Polymorphic Information Content (PIC) of the marker ranged from 0.12 to 0.72 with an average of 0.36 ± 0.05 in the collection (Table S2).

All the isolates from soil of agroecosystem and insect hosts of semi natural habitat were identified to be *B. bassiana*. Three populations were observed in the microsatellite loci examined by multiple allelic differences. ML analysis of the microsatellite genetic structure resolved three phylogenetic species, Bb_1, Bb_2, and Bb_3, with 6, 4, and 5 isolates of *B. bassiana*, respectively (Fig. 2). A total of 7 unique alleles were detected within the three phylogenetic species of *B. bassiana*. The total number of genotypes detected was 12 in the collection. All the three phylogenetic species were detected with 4 genotypes each. The three phylogenetic species resolved in microsatellite genotype analysis concordantly supported the three phylogenetic species resolved in the combined Bloc, TEF, and ITS phylogenetic analysis (Fig. 1).

Pathogenicity of *B. bassiana*

Screening of the isolates of *B. bassiana* against cowpea aphid, *A. craccivora*, using a single concentration of 1×10^6 conidia/ml showed that mortality of aphids ranged

between 46.67 and 100% and pathogenicity varied significantly between isolates ($p < 0.05$) (Table S3). Bioassays of the three highly pathogenic and one less pathogenic isolates of *B. bassiana* against *A. craccivora* showed that mortality of aphids ranged between 20 and 100% for concentrations ranging from 4×10^5 to 1×10^6 conidia/ml (Table 1). Mean per cent mortality of aphid was significantly high (100%) with the highest concentration of 1×10^6 conidia/ml for GKVK 01_13 isolate followed by GKVK 01_06 isolate (90%) on 5 days post treatment (Table 1). The lowest mortality for the highest concentration was obtained with GKVK 01_24 isolate (50%). Mortality varied significantly between concentrations of the isolates ($p < 0.05$) and increased with increase in conidial concentration.

Extracellular chitinase activity

Chitinase activity of *B. bassiana* varied significantly between the four pathogenic isolates on 12 days post inoculation in chitin medium ($p < 0.05$). The highest chitinase activity of 0.554 U/ml was detected with GKVK 01_13 isolate followed by GKVK 01_06 isolate (0.450 U/ml), and the lowest chitinase activity of 0.016 U/ml was detected with GKVK 01_24 isolate (Table 2). Observations on chitinase activity of the isolates of *B. bassiana* were in concordance with the results of pathogenicity of isolates evaluated in bioassays (Table 1).

Fig. 2 Unrooted maximum likelihood tree of microsatellite genotypes for phylogenetic species of *B. bassiana*. Bootstrap values $\geq 70\%$ are listed above relevant internodes

Table 1 Mean per cent mortality of *A. craccivora* infected with *B. bassiana* isolates

Isolate	Control	4×10^5 Conidia/ ml	6×10^5	8×10^5	1×10^6
GKVK 01_06	3.33 (6.15 ± 6.14) ^j	56.67 (48.85 ± 1.92) ^{def}	66.67 (54.78 ± 2.00) ^{de}	83.33 (66.15 ± 2.71) ^b	90 (71.57 ± 0.00) ^b
GKVK 01_13	3.33 (6.15 ± 6.14) ^j	70 (56.79 ± 0.00) ^{cd}	80 (63.43 ± 0.00) ^{bc}	90 (71.57 ± 0.00) ^b	100 (87.13 ± 0.00) ^a
GKVK 01_24	0.00 ^j	20 (26.57 ± 0.00) ⁱ	30 (33.21 ± 0.00) ^{hi}	40 (39.23 ± 0.00) ^{gh}	50 (45.00 ± 0.00) ^{fg}
GKVK 01_31	3.33 (6.15 ± 6.14) ^j	53.33 (46.92 ± 1.92) ^{efg}	63.33 (52.78 ± 2.01) ^{def}	70 (56.79 ± 0.00) ^{cd}	83.33 (66.15 ± 2.71) ^b

Mean values with different superscript letters in each column and row are significantly different; angular transformed mean values with SE are given in parentheses

Discussion

In the present study, the combined use of phylogenetic and microsatellite markers facilitated detailed assessment of genetic diversity within *B. bassiana* distributed in the south part of Karnataka State. Sequence of the intergenic region, Bloc successfully identified the isolates of *B. bassiana* and sequence of the TEF region resolved the isolates to specific clades in phylogenetic tree [6, 7]. Multilocus phylogeny of the isolates based on partial sequences of Bloc, TEF, and ITS revealed the coexistence of three phylogenetic species of *B. bassiana*, Bb_1, Bb_2, and Bb_3, in semi natural and cultivated agricultural habitats at a regional scale, further reinforced by allelic differences in maximum likelihood analysis of multiple microsatellite loci. Coexistence of different genetic groups of the morphologically cryptic species of *B. bassiana* in soils and insect hosts of agricultural and semi natural habitats at a regional scale was demonstrated [9, 13]. Occurrence of similar ephemeral resources in different regions at a local scale could facilitate the coexistence of the multiple phylogenetic species of *B. bassiana*.

It is observed that the 3 phylogenetic species of *B. bassiana* were not associated with any of the two crop plants in agroecosystem or insect hosts belonged to 3 orders in semi natural habitat, instead they appeared as generalist fungal entomopathogens. Multispecies sympatry of *B. bassiana* within sap-sucking insect pest of crops such as tamarind and ber, *O. oenanthus* was observed in all the 3 phylogenetic clades retrieved. Occurrence of sympatric cryptic species of

B. bassiana within single insect host belongs to different insect orders was suggested [6, 9, 13]. In addition, more isolates are required to make a decision on the phylogenetic and taxonomic status of *B. bassiana* population in semi natural and agricultural habitats.

Microsatellite genotypes were determined for *B. bassiana* population occurring in the south part of Karnataka State to understand their genotypic diversity. Seventeen microsatellite markers detected 12 genotypes within 15 isolates of *B. bassiana* and 4 genotypes were identified within each phylogenetic species in the study. This observation parallels the high genotype diversity of *B. bassiana* in different studies using the same SSR markers. Twenty-six genotypes within 33 isolates of *B. bassiana* using 18 markers were detected in soils and insect hosts of agroecosystem and semi natural habitat in Denmark [9]. Twenty genotypes within 82 isolates of *B. bassiana* from emerald ash borer, *Agilus planipennis*, using 7 markers were recorded in Michigan [32]. Seven genotypes within 10 isolates of *B. bassiana* using 15 markers were detected in pollen beetle, *Meligethes aeneus*, in Switzerland [13]. Loci that amplified across the isolates showed high level of polymorphism suggesting the transfer of microsatellite markers among phylogenetic species of *B. bassiana*.

Annual crop sugarcane selected in the study was less exposed and disturbed due to reduced tillage of soil. This could be the reason for increased occurrence of *B. bassiana* in the soil of sugarcane crop. Cultural practices like reduced tillage regimes enhanced *B. bassiana* levels in the soil [33]. Detection of different genotypes in soils of two crop plants and insect hosts of semi natural habitat was supported by high allelic and genotypic diversity in which multilocus genotypes occur in different geographic locations and insect hosts. This indicates that several genotypes of *B. bassiana* were widespread in soils of agroecosystem and insect hosts of semi natural habitat. Similar observation on the occurrence of diverse genotypes of *B. bassiana* in soils and insect hosts of agroecosystem and semi natural habitat in Denmark was suggested [9]. However, extensive geographical surveys of different habitats will help to recognize more genotypes of *B. bassiana*.

Table 2 Chitinase activity of *B. bassiana* isolates

Isolate	Chitinase activity (U/ml)
GKVK 01_06	0.450 ± 0.006 ^b
GKVK 01_13	0.554 ± 0.002 ^a
GKVK 01_24	0.016 ± 0.001 ^d
GKVK 01_31	0.350 ± 0.005 ^c

Mean of three replicates with SE; mean values with different superscript letters are significantly different

High virulence of pathogen is crucial in the biocontrol of insect pests. Evaluation of four isolates of *B. bassiana* against cowpea aphid, *A. craccivora*, revealed that all isolates varied significantly in their pathogenicity. Cow bug, *O. oneratus*, derived isolates of *B. bassiana* were found to be highly pathogenic against *A. craccivora*. This could be due to the taxonomic similarity of the insect host of pathogen with the insect pest evaluated in bioassay, since both insects belong to order Hemiptera. Findings of the extracellular chitinase activity were in concordance with the pathogenicity of the isolates of *B. bassiana* evaluated in bioassays. Therefore, chitinase activity can be correlated with the virulence of pathogen. Endo-type chitinolytic enzyme, chitinase randomly degrade chitin to β -1, 4-linked N-acetyl-D-glucosamine (GlcNAc) units. Strains that are highly pathogenic produced detectable amount of extracellular chitinase, protease, and lipase activity in the cultural filtrate [34]. Chitinase enzyme plays an important role in the pathogenicity of *B. bassiana* against insects [35]. Application of cow bug–derived highly pathogenic isolates of *B. bassiana* against cowpea aphid, *A. craccivora*, would lead to the effective protection of cowpea against aphid attack by significantly reducing the quantity of inoculum needed for application and thus decreasing the cost of crop protection.

The study revealed information on the genetic diversity and biocontrol efficiency of *B. bassiana* that serve as a resource to promote the future work at the local level. Diverse phylogenetic species and genotypes of *B. bassiana* were found to coexist within the insect hosts and soils of semi natural and agricultural habitats, respectively. These pathogens could play an important role in the regulation of insect pest populations in semi natural and agricultural habitats in the Karnataka State of India. Semi natural habitat was detected as the major reservoir of fungal entomopathogen, *B. bassiana*. Application of indigenous virulent strains of *B. bassiana* could provide enhanced control of native insect pest, *A. craccivora*, since they are highly adapted to the particular environment. Further studies on the ecological and insect host adaptations in different environments may provide more essential information on *B. bassiana* for the control of insect pests in agroecosystem.

Acknowledgments I am grateful to advisor late C. K. Suresh for the supervision in research. I thank Stephen A. Rehner, ARS, USDA for his suggestions in the identification of *B. bassiana*. I am thankful for the support of K. Praveen Karanth, CES, IISc, Bangalore in phylogenetic analysis. I thank C. A. Viraktamath, Entomology, UAS, GKVK, who kindly identified the insect hosts of this study.

Funding information The study was financially supported by the Science and Engineering Research Board (SERB) Young Scientist scheme (SR/FT/LS-156/2010) Govt. of India, New Delhi.

Compliance with ethical standards

Conflict of interest The author declares that she has no conflict of interest.

References

1. Ferron P (1978) Biological control of insect pests by entomogenous fungi. *Ann Rev Entomology* 23:409–422
2. Steinhaus EA (1963) Insect pathology: an advanced treatise. Academic Press. 689 p, New York
3. Keller S, Zimmermann G (1989) Mycopathogens of soil insects. In: Wilding N, Collins NM, Hammond PM, Webber JF (eds) *Insect-Fungus Interactions*. Academic Press, pp 239–270
4. Goettel MS, Paprawsk TJ, Vendenburg JD, Li Z, Roberts DW, Laird M (1990) Safety to nontarget invertebrates of fungal biocontrol agents. In: Laird M, Lacey LA, Davidson EM (eds) *Safety of microbial insecticides*. CRC Press, Boca Raton, FL, pp 209–231
5. Inglis GD, Goettel MS, Butt TM, Strasser H (2001) Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N (eds) *Fungi as biocontrol agents*. CAB international, Wallingford, pp 23–69
6. Rehner SA, Posada F, Buckley EP, Infante F, Castillo A, Vega FE (2006) Phylogenetic origins of African and Neotropical *Beauveria bassiana* pathogens of the coffee berry borer, *Hypothenemus hampei*. *J Invertebr Pathol* 93:11–21. <https://doi.org/10.1016/j.jip.2006.04.005>
7. Rehner SA, Buckley EP (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence of cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97:84–96. <https://doi.org/10.3852/mycologia.97.1.84>
8. Rehner SA, Minnis AM, Sung GH, Luangsa-ard JJ (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. *Mycologia* 103:1055–1073. <https://doi.org/10.3852/10-302>
9. Meyling NV, Lübeck M, Buckley EP, Eilenberg J, Rehner SA (2009) Community composition, host range, and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and semi-natural habitats. *Mol Ecol* 18:1282–1293. <https://doi.org/10.1111/j.1365-294X.2009.04095.x>
10. Meyling NV, Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. *Biol Control* 43:145–155. <https://doi.org/10.1016/j.biocontrol.2007.07.007>
11. Shah PA, Pell JK (2003) Entomopathogenic fungi as biological control agents. *Appl Microbiol Biotechnol* 61:413–423. <https://doi.org/10.1007/s00253-003-1240-8>
12. Bidochka MJ, Menzies FV, Kamp AM (2002) Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. *Arch Microbiol* 178: 531–537. <https://doi.org/10.1007/s00203-002-0490-7>
13. Meyling NV, Pilz C, Keller S, Widmer F, Enkerli J (2012) Diversity of *Beauveria* spp. isolates from pollen beetles *Meligethes aeneus* in Switzerland. *J Invertebr Pathol* 109:76–82. <https://doi.org/10.1016/j.jip.2011.10.001>
14. Agrawal Y, Mual P, Shenoy BD (2014) Multi-gene genealogies reveal cryptic species *Beauveria rudraprayagi* sp. nov from India. *Mycosphere* 5:719–736. <https://doi.org/10.5943/mycosphere/5/6/3>
15. Neelapu NR, Reineke A, Chanchala UM, Koduru UD (2009) Molecular phylogeny of asexual entomopathogenic fungi with special reference to *Beauveria bassiana* and *Nomuraea rileyi*. *Rev Iberoam Micol* 26:129–145. [https://doi.org/10.1016/S1130-1406\(09\)70024-5](https://doi.org/10.1016/S1130-1406(09)70024-5)
16. Blackman RL, Eastop VF (2007) Taxonomic issues. In: Van Emden HF, Harrington R (eds) *Aphids as crop pests*. CAB International, Wallingford, pp 1–29
17. Ramanujam B, Poornesha B, Dileep RC, Japur K (2017) Field evaluation of entomofungal pathogens against cowpea aphid, *Aphis craccivora* Koch and their effect on two coccinellid

- predators. *Int J Pest Manage* 63:101–104. <https://doi.org/10.1080/09670874.2016.1227881>
18. Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Zhang S, Keyhani NO (2013) Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: hydrocarbon oxidation within the context of a host-pathogen interaction. *Front Microbiol* 4(24). <https://doi.org/10.3389/fmicb.2013.00024>
 19. Pedrini N, Crespo R, Juárez MP (2007) Biochemistry of insect epicuticle degradation by entomopathogenic fungi. *Comp Biochem Physiol C: Toxicol Pharmacol* 146:124–137. <https://doi.org/10.1016/j.cbpc.2006.08.003>
 20. Moraes CKD, Schrank A, Vainstein MH (2003) Regulation of extracellular chitinases and proteases in the entomopathogen and acaricide *Metarhizium anisopliae*. *Curr Microbiol* 46:205–210
 21. Goettel MS, St Leger RJ, Rizzo NW, Staples RC, Roberts DW (1989) Ultrastructural localization of a cuticle-degrading protease produced by the entomopathogenic fungus *Metarhizium anisopliae* during penetration of host (*Manduca sexta*). *J Gen Microbiol* 135: 2233–2239
 22. Zimmermann G (1986) The ‘*Galleria* bait method’ for detection of entomopathogenic fungi in soil. *J Appl Entomol* 102:213–215. <https://doi.org/10.1111/j.1439-0418.1986.tb00912.x>
 23. Cullings KW (1992) Design and testing of a plant specific PCR primer for ecological and evolutionary studies. *Mol Ecol* 1:233–240. <https://doi.org/10.1111/j.1365-294X.1992.tb00182.x>
 24. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322
 25. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739. <https://doi.org/10.1093/molbev/msr121>
 26. Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Org Divers Evol* 12:335–337. <https://doi.org/10.1007/s13127-011-0056-0>
 27. Rehner SA, Buckley EP (2003) Isolation and characterization of microsatellite loci from the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales). *Mol Ecol Notes* 3:409–411. <https://doi.org/10.1046/j.1471-8286.2003.00464.x>
 28. Yeh FC, Yang RC, Boyle T (1999) POPGENE version 1.32; Microsoft Windows based freeware for population genetic analysis. Center for International Forestry Research, University of Alberta, Edmonton
 29. Rohlf FJ (1998) NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.02. Applied Biostatistics Inc. Exeter Publishing, Setauket
 30. SPSS Inc (2008) SPSS Statistics for Windows, Version 17.0. SPSS Inc, Chicago
 31. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428. <https://doi.org/10.1021/ac60147a030>
 32. Castrillo LA, Bauer LS, Liu H, Griggs MH, Vandenberg JD (2010) Characterization of *Beauveria bassiana* (Ascomycota: Hypocreales) isolates associated with *Agrilus planipennis* (Coleoptera: Buprestidae) populations in Michigan. *Biol Control* 54:135–140. <https://doi.org/10.1016/j.biocontrol.2010.04.005>
 33. Hummel RL, Walgenbach JF, Barbercheck ME, Kennedy GG, Hoyt GD, Arellano C (2002) Effects of production practices on soil-borne entomopathogens in western North Carolina vegetable systems. *Environ Entomol* 31:84–91. <https://doi.org/10.1603/0046-225X-31.1.84>
 34. Samuels KDZ, Heale JB, Llewellyn M (1989) Characteristics relating to the pathogenicity of *Metarhizium anisopliae* toward *Nilaparvata lugens*. *J Invertebr Pathol* 53:25–31
 35. Havukkala I, Mitamura C, Hara S, Hirayae K, Nishizawa Y, Hibi T (1993) Induction and purification of *Beauveria bassiana* chitinolytic enzymes. *J Invertebr Pathol* 61:97–102

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