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# Isolation, molecular characterization of indigenous *Beauveria bassiana* isolate, using ITS-5.8 s rDNA region and its efficacy against the greatest wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a model insect

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## Abstract

The entomopathogenic fungi (EPF), as a biological control agent, can provide an alternative option for high-risk insecticides. *Beauveria bassiana* is one of the most potential EPF of pest control all over the world. The EPF isolate Y-F\_ITS1 was isolated from Egyptian fauna on *Beauveria*-specific selective medium (BS medium). Molecular screening of the *B. bassiana* isolate, using PCR amplification with *B. bassiana*-specific primers and nucleotide sequence analysis of the internal transcribed spacer (ITS) region, confirmed the isolate as *B. bassiana* (accession no. [MK773644.1]). Efficacy of the isolate Y-F\_ITS1 was examined against fourth instar larvae of *Galleria mellonella* L. as a model insect using concentrations from  $10^4$  to  $10^7$  spores  $\text{ml}^{-1}$  in a contact toxicity assay under laboratory conditions. The pathogenicity experiment showed that all the tested concentrations caused mortalities ranged from 75 to 98.33%, within 5 days posttreatment with cumulative mortalities, reached 100%, before 7 days with typical symptoms of infection and sporulation. Further investigations are needed to prove its efficacy against different economic pests as a credible candidate of integrated pest management (IPM) program.

**Keywords:** Biological control, Entomopathogenic fungi, *Beauveria bassiana*, ITS, *Galleria mellonella*

## Background

There is an increasing expectancy worldwide in developing microbial bio-pesticides as a considerable component of integrated pest management programs (IPM) and stimulated necessities for chemical-free pesticides (Ravensberg 2015). The entomopathogenic fungi (EPF) fulfilled the criteria as a promising natural mortality factor of insects (Boomsma et al. 2014). *Beauveria* spp. based products proved their virulence and represent almost about 40% of the total myco-insecticides (Faria and

Wraight 2007). Quite recently, considerable attention has been paid to the use of DNA barcoding, using the internal transcribed spacer (ITS), as a short gene sequence was taken from a standardized portion of the genome and can be recovered and characterized as a unique identification marker for all biological identifications and diagnostic species including fungi (Hebert and Barrett 2005; and Pečnikar and Buzan 2014). Nearest neighbor algorithms are usually used to assign an unknown sample to a known species by finding the closest sequence to the sample sequence in the international database (Saitou and Nei 1987). Basic Local Alignment Search Tool (BLAST) is a common matching tool,

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provided through the National Center of Biotechnology Information (NCBI) that searches for correspondence between a query sequence and a sequence library (Altschul 1990). The main target of this study was to identify a new EPF isolate from Egyptian fauna and evaluate its efficacy against the greatest wax moth, *Galleria mellonella* L., as a model insect under laboratory conditions.

## Materials and methods

### Sample collection

Various clay soil samples from different clover farm locations at Dakahlia Governorate, Egypt, were collected by gathering the topsoil down to 40 cm depth, using metal shovel in late winter 2017. The samples of each site were placed in sterile plastic bags, transferred to the laboratory, and stored at 4–8 °C until been used (Chandler et al. 1997).

### Insect bait method

Insect bait technique recommended by (Zimmermann 1986) was chosen to screen and isolate the indigenous species of EPF, using larvae of the wax moth, *G. mellonella*. Larvae were treated by warm water to prevent extensive webbing in the soil (Meyling and Eilenberg 2006). Soil samples were moistened and placed in petri dishes. Twenty medium-sized larvae were used for each soil sample. Samples were incubated at  $20 \pm 2$  °C in the dark and inverted every day. Soil samples were examined after 5 days; dead bait larvae were collected and surface sterilized with 1% Na-hypochlorite to prevent external saprophytic fungi from growing on the dead cadaver. Dead larvae were kept in petri dish lined with a single layer of wet filter paper until signs of muscardine were observed. The fungal spore was cultivated on Sabouraud dextrose yeast agar (SDAY) medium. The petri dishes (5 cm × 1 cm) were incubated at 25 °C for 3–7 days. For extra purification, single spore cultures were plated out from multispore cultures. Fungal strain exhibiting good growth and spore production traits was selected, purified, and identified according to microscopic observations following the taxonomic keys, using color atlas of pathogenic fungi for *Beauveria* genus (Frey et al. 1979; Webster and Weber 2007).

### Molecular identification of fungal isolate

Mycelia and conidia from the isolated fungal strain were inoculated, and a single spore colony was grown on SDAY and incubated on a shaker (150 rpm) at 20 °C for 5–7 days. DNA extraction was done, following the manufacturer's protocol, using Animal and Fungi DNA Preparation Kit (Jena Bioscience, Germany). Thermo Scientific NanoDrop Lite Spectrophotometer (Thermo Scientific, Waltham, MA, USA) determined the quantity and quality of DNA. For the ITS1-5.8S-ITS2, PCR

amplification and sequencing were performed. ITS1F (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4R (5'-TCC TCC GCT TAT TGA TAT GC-3') primers were used to amplify the partial gene region. Using ABI Prism Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA), amplicons were sequenced with 310 Automated DNA Sequencer (Company, country). A BLASTN search was conducted on the NCBI database to classify associated sequences. The BioEdit 4.8.9 software alignment editor (Hall 1999) was used and the alignment was corrected manually. CLUSTAL-X was used to perform multiple sequences alignment. A phylogenetic tree was reconstructed using MEGA ver. 7.0 by using Maximum Likelihood method based on the Jukes-Cantor model. Branch support was estimated with 1000 bootstrap replicates under appropriate substitution models. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer phylogenetic tree.

### Efficacy of the fungal isolate against *G. mellonella*

Fourth instar larvae of *G. mellonella* were used in the present experiment. For treatment experiments and control, 30 larvae (10 larvae per replicate) were placed in plastic boxes (25 × 25 × 20 cm; length × width × height). All boxes were provided by a sterile wax for nutrition. To determine the LC<sub>50</sub> and LC<sub>90</sub>, 5 spore concentrations of the fungal suspension were used (viz. 10<sup>7</sup>, 10<sup>6</sup>, 10<sup>5</sup>, 10<sup>4</sup>, and 10<sup>3</sup> spores ml<sup>-1</sup>). For each concentration, 30 larvae were placed on a folded Whatman No. 1 filter paper in a glass funnel, and then 30 ml of the fungal suspensions were added; sterile distilled water mixed with 0.1% Tween 20 was used as a control treatment. The treated insects were left for 30 s to dry and then transferred to the plastic boxes lined with a filter paper to remove excess humidity. All treatments and the control were incubated at 25 °C. The whole experiment was repeated 3 times. The mortality rate was recorded daily for 6 days.

### Statistical analysis

The mortality percentage caused by EPF was corrected according to Abbott's formula (Abbott 1925). Percentage mortality was calculated according to Feng et al. (1992). The lethal time LT<sub>50</sub> and LT<sub>90</sub> values, that were required to kill 50 and 90% respectively, were estimated according to Finney (1971), using the "LdPLine" software to the [http://embakr.tripod.com/ldpline/ldpline.htm].

## Results and discussion

### Isolation and morphological identification

After 5 days of soil sample examination, the dead bait larvae of *G. mellonella* were separated, sterilized, and

kept in a petri dish until infection symptoms appear. The infection test showed that the dead bait larvae suffered infection symptoms, feed less actively, slow motion, and sluggishness. White mycelium grew in membranes and inter-segmental membranes of dead larvae after 5 days or longer. Morphological identification indicated that the strain Y-F ITS1 was *B. bassiana*. The samples collected fungal species on selective BS medium showed morphological and cultural characteristics similar to *Beauveria* species. Colonies were lightly raised, round, with white powdery surface, and lightly downy with circular rings. White powdery translucent mycelium showed a radial growth. Conidiospores were densely clustered in whorls, one celled, smooth, hyaline, and short. The isolation of EPF, using *Galleria* bait method, proved to be an effective technique for screening indigenous species than common plating on media. Keller et al. (2003) and Meyling and Eilenberg (2006) mentioned that isolation and identification, using baiting soil samples with larvae of *G. mellonella*, could be used on a broad range for indigenous isolates present in soil. Identification based on morphological characteristics could not identify correctly down to the species level;

thus, the genetic diversity of *Beauveria* species were detected, using ITS and additional loci such as EF- $\alpha$  or mt intergenic regions (Aquino de Muro et al. 2005; Rehner and Buckley 2005; Ghikas et al. 2010; Poëaim et al. 2014; and Chen et al. 2018).

#### Molecular characterization

A total of 1597 bp was deposited under accession no. MK773644.1 in the GenBank with 73.48% as GC content of the present sordariomycetes isolate for the ITS1-5.8S-ITS2 gene region. A unique genetic sequence was revealed by a parallel comparison of the isolated gDNA sequence of the present isolate with a range of other genotypes belonged to Hypocreomycetidae subclass. Calculating the percentage of identity between this novel sequences and the others from GenBank revealed a high degree of resemblance (> 90%). Phylogenetic analyses were conducted based on the alignment of partial and complete sequences of ITS1-5.8S-ITS2 for 24 taxa using Maximum Likelihood method based on the Jukes-Cantor model representing 2 orders, Hypocreales and Glomerellales (Table 1 and Fig. 1).

**Table 1** Species used in the phylogenetic analysis of ITS1 gene region of the present *Beauveria bassiana*

Name of isolates	Order/family	Isolate source	Accession no.	GC content %	Identity %
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	<i>Argania spinosa</i> forest soil	KT378247.1	56.52	99.61
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	<i>Galleria mellonella</i>	KU523254.1	55.82	99.42
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	<i>Coffea Arabica</i>	DQ682566.1	55.68	99.42
<i>Beauveria pseudobas</i>	Hypocreales/Cordycipitaceae	Agricultural soil	KC355185.1	56.36	99.41
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	Farm soil	MH233319.1	56.57	99.41
<i>Isaria farinose</i>	Hypocreales/Cordycipitaceae	<i>Bombyx mori</i>	JF429897.1	50.21	99.31
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	Ant	KX553851.1	54.46	99.23
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	<i>Cycloneda sanguinea</i>	KF308683.1	55.82	99.23
<i>Cordyceps bassiana</i>	Hypocreales/Cordycipitaceae	<i>Coffea arabica</i>	EF672309.1	55.78	99.23
<i>Cordyceps</i> sp.	Hypocreales/Cordycipitaceae	<i>Miscanthus giganteus</i>	HQ630968.1	55.24	99.23
<i>Beauveria</i> sp.	Hypocreales/Cordycipitaceae	Marine sponge	JQ922139.1	54.99	99.22
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	–	MH233302.1	56.38	98.85
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	<i>Hypothenemus hampei</i>	MK049981.1	55.96	98.85
<i>Beauveria</i> sp.	Hypocreales/Cordycipitaceae	–	JQ411373.1	54.72	97.97
<i>Beauveria bassiana</i>	Hypocreales/Cordycipitaceae	Punjab soil	MG670098.1	55.22	97.78
<i>Lecanicillium</i> sp.	Hypocreales/Cordycipitaceae	<i>Ophiocordyceps sinensis</i>	JF794475.1	51.24	94.51
<i>Fusarium proliferatum</i>	Hypocreales/Nectriaceae	–	LT841264.1	51.82	93.21
<i>Fusarium solani</i>	Hypocreales/Nectriaceae	–	FJ345352.1	52.03	92.26
<i>Fusarium oxysporum</i>	Hypocreales/Nectriaceae	–	LT841236.1	51.65	92.87
<i>Epichloe festucae</i>	Hypocreales/Clavicipitaceae	–	KU710346.2	52.67	93.18
<i>Metarhizium anisopliae</i>	Hypocreales/Clavicipitaceae	–	AF218207.1	51.10	92.10
<i>Clonostachys rosea</i>	Hypocreales/Bionectriaceae	–	MH047188.1	50.38	92.80
<i>Acremonium</i> sp.	Hypocreales/Incertae sedis	–	GQ867783.1	52.20	92.27
<i>Colletotrichum graminicola</i>	Glomerellales/Glomerellaceae	–	XR 001139481.1	51.68	92.30



**Fig. 1** Sequence alignment of ITS1 gene region of the present *Beauveria bassiana* with the most closely related species. Only variable sites are shown. (.) represents bases identical to those of the first sequence and (-) indicates gaps

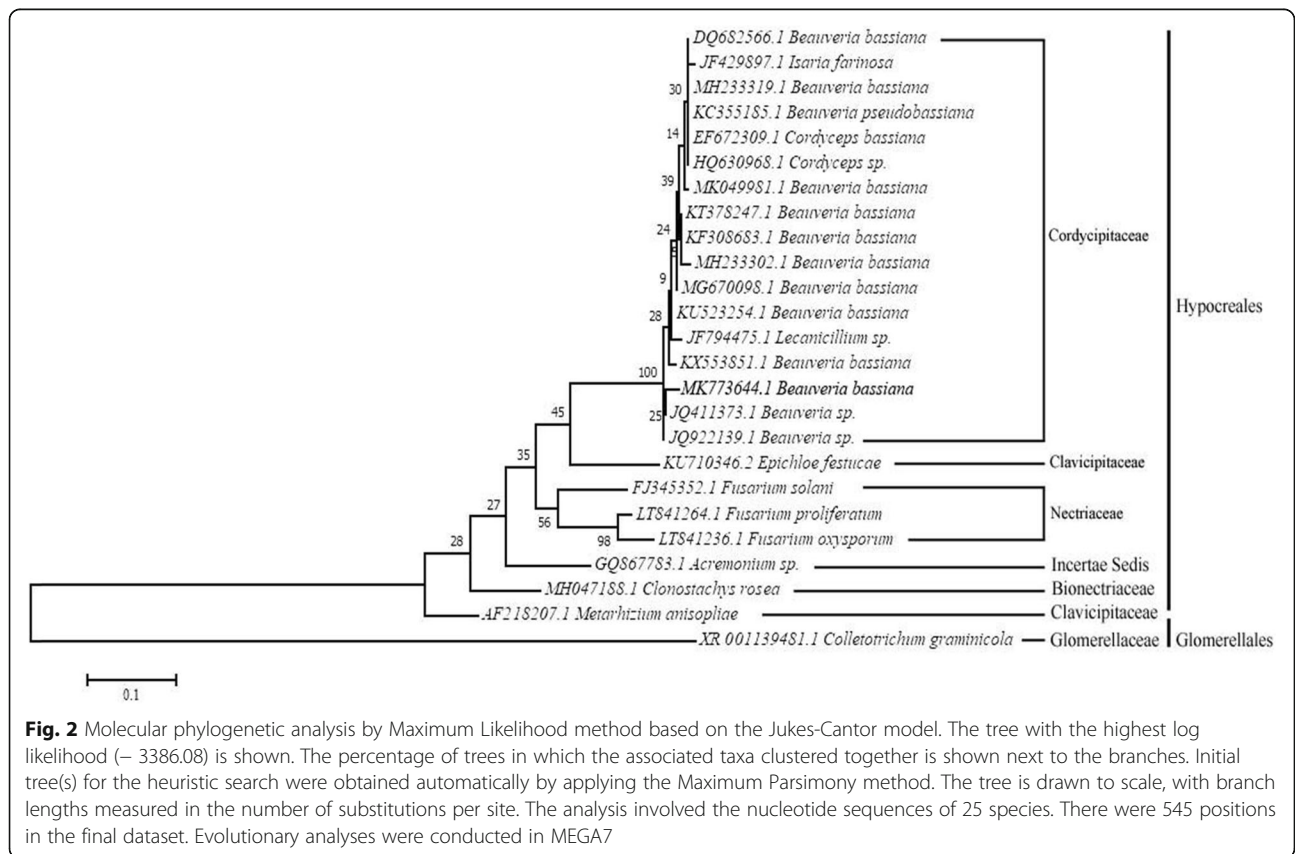
Comparison of the nucleotide sequences and divergence showed that the ITS1-5.8S-ITS2 of this isolate revealed sequence identities of 92.27–99.61% with members of the order Hypocreomycetidae and 92.30% of the Glomerellales (Table 1). Among Hypocreomycetidae, the present isolate showed 94.51–99.61% sequence identity with taxa under

Cordycipitaceae, 92.10–93.18% with Clavicipitaceae, 92.26–93.21% with Nectriaceae, 92.27% with Incertae sedis, and 92.80% with Bionectriaceae. Among Cordycipitaceae, the maximum sequence identity (99.61%) with lowest divergent value was recorded between the present isolate and *B. bassiana* (gb KT378247.1), followed by *B. bassiana* (99.42%, gb

KU523254.1, DQ682566.1), *B. pseudobassiana* (99.42%, gb KC355185.1), *B. bassiana* (99.41%, MH233319.1), *Isaria farinose* (99.31%, gb JF429897.1), *B. bassiana* (99.23%, gb KX553851.1, KF308683.1), *Cordyceps bassiana* (99.23%, gb EF672309.1), *Cordyceps* sp. (99.23%, gb HQ630968.1), *Beauveria* sp. (99.22%, gb JQ922139.1), *B. bassiana* (98.85%, gb MH233302.1, MK049981.1), *Beauveria* sp. (97.97%, gb JQ411373.1), *B. bassiana* (97.78%, gb MG670098.1), and *Lecanicillium* sp. (94.51%, gb JF794475.1).

The ME tree showed a cluster that contained all of the sordariomycetes taxa, which was clearly divided into 2 distinct clades (Fig. 1). The first clade divided into 2 subclades, one clustered all the contained Cordycipitaceae taxa, while the other contained other taxa of the Clavicipitaceae, Nectriaceae, Incertae sedis, and Bionectriaceae. The second clade, represented by *Colletotrichum graminicola* belonged to the Glomerellaceae within the Glomerellales. The ME tree showed a well-resolved distinct clade for the present isolate with members belonged to the family Cordycipitaceae and deeply embedded within the genus *Beauveria* with close relationship to the previously described *B. bassiana* (gb KT378247.1, KU523254.1, DQ682566.1, MH233319.1, KX553851.1,

KF308683.1, MH233302.1, MK049981.1, and MG670098.1) as a more related sister taxa (Fig. 2). In the present study, the internal transcribed spacer region (ITS1-5.8S-ITS2) was selected in agreement with White et al. (1990), Curran et al. (1994), and Zare and Gams (2001) who reported that the nuclear ITS1-5.8S-ITS2 region was identified as a useful tool for providing molecular sequence data suitable for establishing fungal phylogenies. Herein, the selected gene region was amplified by PCR, using the ITS1F/4R primers as recommended by White et al. (1990) who considered them as universal primers for fungal DNA barcode. The ML analysis of the DNA-ITS region formed a highly supported clade together with other isolates retrieved from the GenBank. The clustering of the recovered *B. bassiana* isolate showed the closest relationship with different isolates of *B. bassiana*, *B. pseudobassiana*, and *Beauveria* sp. from other regions. The obtained phylogeny supports the monophyly of the *Beauveria* group with low variation observed among these ITS regions, which agree with Coates et al. (2002), followed by Ghikas et al. (2010) who supported the observation that other genetic variant are likely to occur more rapidly than mutations detected in ITS regions. The presence of *B. pseudobassiana* in the present



**Fig. 2** Molecular phylogenetic analysis by Maximum Likelihood method based on the Jukes-Cantor model. The tree with the highest log likelihood (-3386.08) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved the nucleotide sequences of 25 species. There were 545 positions in the final dataset. Evolutionary analyses were conducted in MEGA7

**Table 2** Efficacy of the *B. bassiana* strain (Y-F ITS1) against fourth instar larvae of *G. mellonella*

Concentrations (spores ml <sup>-1</sup> )	24 hours	48 hours	72 hours	96 hours	120 hours	LT <sub>50</sub> (days)	LT <sub>90</sub> (days)	
	Mortality (%)							
1 × 10 <sup>4</sup>	0.8 ± 0.5	3.3 ± 0.5	6.6 ± 0.5	50 ± 1	75 ± 1	–	–	
1 × 10 <sup>5</sup>	1.66 ± 0.5	5.8 ± 0.5	26.6 ± 0.5	68.3 ± 0.5	80 ± 1	3.58	5.91	
1 × 10 <sup>6</sup>	1.66 ± 0.5	10 ± 1	35 ± 1	83 ± 1	93.6 ± 1.5	–	–	
1 × 10 <sup>7</sup>	8.33 ± 1	26.6 ± 0.15	51.66 ± 0.5	90 ± 1	98.3 ± 0.5	–	–	

phylogeny with *B. bassiana* assured the hypothesis of Rehner et al. (2011) that although they comprise morphospecies group, the phylogenetic analysis demonstrated that both of them are distantly related. In addition, Entz et al. (2005), Becerra et al. (2007), Freed et al. (2011), and Muthabathula et al. (2019) supposed that the *B. bassiana* and *Cordyceps militaris* are closely related, which share the same clade in agreement with the present results.

#### Efficacy of the *B. bassiana* strain against *G. mellonella*

As shown in Table 2, *G. mellonella* larvae exhibited considerable linear mortality percentages when inoculated with serial concentrations (viz. 10<sup>7</sup>, 10<sup>6</sup>, 10<sup>5</sup>, and 10<sup>4</sup> spores ml<sup>-1</sup>) of the indigenous *B. bassiana* strain Y-F ITS1 that was isolated from Egyptian fauna. The highest concentration (1 × 10<sup>7</sup> spores ml<sup>-1</sup>) showed mortality percentages ranged from 8.33 to 98.33% after 24 and 120 h, respectively, while the least concentration (1 × 10<sup>4</sup>) spores ml<sup>-1</sup> showed mortality percentages ranged from 0.8 to 75% after 24 and 120 h, respectively. It was also noticed that all the concentrations used in this investigation resulted in 100% mortality of the treated larvae within 7 days. Typical symptoms could be developed on the infected larvae when kept under high humidity in petri dishes within 15 days with a complete sporulation. Concerning the lethal time LT<sub>50</sub> and LT<sub>90</sub> values (calculated for the mortality ranged between 26.6–80%) were 3.58 days and 5.91 days, respectively for *G. mellonella* larvae inoculated with the concentration 1 × 10<sup>5</sup> spores ml<sup>-1</sup>. The obtained results using different concentration of fungal spores indicated that the mortality rate was directionally proportional to spore concentration and exposure times. These results are in line with Fuguet and Vey (2004) and Shoukry et al. (2019). The highest virulence of this indigenous isolate advocates that it could be part of the biological control systems of IPM programs for the control of insect pests and offers the advantage of no pollution, safety, and avoiding pesticide resistance. Further research is needed to examine its efficacy and large-scale application as a biological control agent of economic pests in agriculture in Egypt.

#### Conclusion

The local fungal isolate of this study, identified as Y-F ITS1, using ITS-5.8 s rDNA region deposited in GenBank, showed a significant pathogenicity towards *G. mellonella*, as a model insect. Further studies are needed to evaluate its efficacy as a biological control agent against economic pests in Egypt.

#### Abbreviations

IPM: Integrated pest management; *B. bassiana*: *Beauveria bassiana*; B.S medium: *Beauveria*-specific selective medium; ITS: Internal transcribed spacer; BLAST: Basic local alignment search tool; NCBI: National Center of Biotechnology Information; *G. mellonella*: *Galleria mellonella*; (SDAY) medium: Sabouraud dextrose yeast agar; LC<sub>50</sub>: Median lethal concentration; LT<sub>50</sub>: Median lethal time; *B. pseudobassiana*: *Beauveria pseudobassiana*

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#### Authors' contributions

All authors contributed significantly to this research and preparation of the manuscript. YF carried out the experiments. YF and RSY analyzed the data; YF was involved in the conception and design of the study. YF, methodology and RSY and YF, writing and editing. All authors read and approved the final manuscript.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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## References

- Abbott WS (1925) A method for computing the effectiveness of insecticides. *J Econ Entomol* 18:265–267. <https://doi.org/10.1093/jee/18.2.265a>
- Altschul SF (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Aquino de Muro M, Elliott S, Moore D, Parker BL, Skinner M, Reid W, El M Bouhssini (2005) Molecular characterization of *Beauveria bassiana* isolates obtained from overwintering sites of Sunn Pest (*Eurygaster* and *Aelia* species). *Mycol Res* 109:294–306. Doi:<https://doi.org/10.1017/S0953756204001832>
- Becerra V, Paredes M, Rojo C, France A, Franco J (2007) Intraspecific differentiation of Chilean isolates of the entomopathogenic fungi *Metarhizium anisopliae* var. *anisopliae* as revealed by RAPD, SSR and ITS markers. *Genet Mol Biol* 30:89–99. <https://doi.org/10.1590/S1415-47572007000100017>
- Boomsma JJ, Jensen AB, Meyling NV, Eilenberg J (2014) Evolutionary interaction networks of insect pathogenic fungi. *Annu Rev Entomol* 59:467–485. <https://doi.org/10.1146/annurev-ento-011613-162054>
- Chandler D, Hay D, Reid AP (1997) Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. *Appl Soil Ecol* 5:133–141. [https://doi.org/10.1016/S0929-1393\(96\)00144-8](https://doi.org/10.1016/S0929-1393(96)00144-8)
- Chen WH, Man L, Huang ZX, Yang GM, Han YF, Liang JD, Liang ZQ (2018) *Beauveria majiangensis*, a new entomopathogenic fungus from Guizhou, China. *Phytotaxa* 333:243–250. <https://doi.org/10.11646/phytotaxa.333.2.8>
- Coates BC, Hellmich RL, Lewis LC (2002) *Beauveria bassiana* haplotype determination based on nuclear rDNA internal transcribed spacer PCR-RELP. *Mycol Res* 106(1):40–50. <https://doi.org/10.1017/S0953756201005305>
- Curran J, Driver F, Ballard JWO, Milner RJ (1994) Phylogeny of *Metarhizium*: sequence analysis of the internally transcribed and 5.8 s region of the ribosomal DNA repeat. *Mycol Res* 9:547–552. [https://doi.org/10.1016/S0953-7562\(09\)80478-4](https://doi.org/10.1016/S0953-7562(09)80478-4)
- Entz SC, Johnson DL, Kawchuk LM (2005) Development of a PCR based diagnostic assay for the specific detection of the entomopathogenic fungus *Metarhizium anisopliae* var. *acidum*. *Mycol Res* 109:1302–1312. <https://doi.org/10.1017/S0953756205003746>
- Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biol Control* 43:237–256. <https://doi.org/10.1016/j.biocontrol.2007.08.001>
- Feng MG, Nowierski M, Johnson JB, Poprawski TJ (1992) Epizootics caused by entomophthorean fungi (Zygomycetes: Entomophthorales) in populations of cereal aphids (*Hom.*, Aphididae) in irrigated small grains of southwestern Idaho, USA. *J Appl Entomol* 113:376–390. <https://doi.org/10.1111/j.1439-0418.1992.tb00678.x>
- Finney DJ (1971) Probit analysis 3rd ed., Cambridge University press, 32 E. 57<sup>th</sup> St., New York, Ny 10022, xv + 333 pp. Doi:<https://doi.org/10.1002/jps.2600600940>
- Freed S, Jin FL, Ren SX (2011) Determination of genetic variability among the isolates of *Metarhizium anisopliae* var. *anisopliae* from different geographical origins. *World J Microbiol Biotechnol* 27:359–370. <https://doi.org/10.1007/s11274-010-0466-8>
- Frey D, Oldfield RJ, Bridger RC (1979) A color atlas of pathogenic fungi. Wolfe Medical publication, Holland, 168p
- Fuguet R, Vey A (2004) Comparative analysis of the production of insecticidal and melanizing macromolecules by strains of *Beauveria* spp.: in vivo studies. *J Invertebr Pathol* 85(3):152–167. <https://doi.org/10.1016/j.jip.2004.03.001>
- Ghikas DV, Kouvelis VN, Typas MA (2010) Phylogenetic and biogeographic implications inferred by mitochondrial intergenic region analyses and ITS1-5.8S-ITS2 of the entomopathogenic fungi *Beauveria bassiana* and *B. brongniartii*. *BMC Microbiol* 10:174. <https://doi.org/10.1186/1471-2180-10-174>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98. [https://doi.org/10.14601/Phytopathol\\_Mediterr-14998u1.29](https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29)
- Hebert PDN, Barrett RDH (2005) Reply to the comment by L. Prendini on 'Identifying spiders through DNA barcodes'. *Can J Zool* 83:505–506. <https://doi.org/10.1139/z05-026>
- Keller S, Kessler P, Schweizer C (2003) Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metarhizium anisopliae*. *Biocontrol* 48:307–319
- Meyling NV, Eilenberg J (2006) Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. *Agric Ecosyst Environ* 113:336–341. <https://doi.org/10.1016/j.agee.2005.10.011>
- Muthabathula P, Koduru UD, Saragadam S (2019) Comparative mitochondrial genome analysis of Hypocrealean fungi with different lifestyles. *Res J Life Sci Bioinform Pharm Chem Sci* 5(1):597–612. <https://doi.org/10.26479/2019.0501.50>
- Pečňikar ZF, Buzan EV (2014) 20 years since the introduction of DNA barcoding: from theory to application. *J Appl Genet* 55:43–52. <https://doi.org/10.1007/s13353-013-0180-y>
- Poeaim S, Hangtrakul S, Soyong K (2014) Genetic diversity of *Beauveria* sp. isolated in Thailand. *Int J Agric Technol* 10(3):607–616
- Ravensberg W J (2015) Crop protection in 2010: towards a natural, efficient, safe and sustainable approach. *Int. Symposium on Biopestic Swansea Univ UK*, pp. 7–9.
- Rehner SA, Buckley EP (2005) A *Beauveria* phylogeny inferred from nuclear ITS and *ef1- $\alpha$*  sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97:84–98. <https://doi.org/10.1080/15572536.2006.11832842>
- Rehner SA, Minnis AM, Sung G-H, Luangsa-Ard JJ, Devotto L, Humber RA (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. *Mycologia* 103(5):1055–1073. <https://doi.org/10.3852/10-302>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Shoukry IF, Ahmed FA, Khater KS, El-lakwah SF, Abd-Elmonem HM (2019) Evaluation of the effectiveness of some entomopathogenic fungi on the greater wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Galleriidae). *Egypt Acad J Biol Sci (A Entomology)* 12(4):41–55. <https://doi.org/10.21608/eajbsa.2019.42990>
- Webster J, Weber RWS (2007) Introduction to fungi. 3rd Edition, Cambridge University Press. Doi: <https://doi.org/10.1017/CBO9780511809026>
- White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. In PCR Protocols: a guide to methods and applications (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 15–322. Academic Press San Diego.
- Zare R, Gams W (2001) A revision of *Verticillium* sect. *Prostrata* IV The genera *Lecanicillium* and *Simplicillium* gen nov *Nova Hedwigia* 73(1):1–50
- Zimmermann G (1986) The *Galleria* bait method for detection of entomopathogenic fungi in soil. *J Appl Entomol* 102:213–215

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