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Isolation and identification of *Metarhizium guizhouense* Xct1 and its pathogenicity to *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Abstract

Background The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of the most destructive invasive pests causing serious damage to maize crops in China. At present, the use of chemical pesticides is the main method to prevent and control *S. frugiperda*. However, the excessive use of chemical pesticides not only causes toxicity in humans and animals but also pollutes the environment and causes public harm. In this context, the present study aimed to isolate, identify and evaluate the pathogenicity of the native strain of *Metarhizium guizhouense* against eggs, larvae and pupae of *S. frugiperda* under laboratory conditions.

Results The fungal isolate was identified as *M. guizhouense* (Xct1) based on its morphological and molecular characteristics by sequencing ITS, β-tubulin and EF1-α gene. Meanwhile, the fungal isolate was highly pathogenic to eggs of the *S. frugiperda*, at the highest concentration of 1×10^8 spores/ml, the cumulative egg hatching rate was only 8.33% at 10 days post exposure. After 10 days of exposure to the highest concentration of 1×10^8 spores/ml, the LC_{50} values for the first–fifth larval instars of *S. frugiperda* were 8.12×10^4, 1.11×10^5, 5.55×10^5, 3.87×10^6 and 1.43×10^7 spores/ml, respectively.

Conclusions The pathogenicity of *M. guizhouense* Xct1 to *S. frugiperda* larvae gradually decreased with increasing the developmental stages. *Metarhizium guizhouense* Xct1 was more pathogenic to egg and younger larvae of *S. frugiperda*. This provides a basis for determining the optimal period of control.

Keywords *Spodoptera frugiperda*, Entomopathogenic fungi, *Metarhizium guizhouense*, Pathogenicity, Mortality

Background

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), belongs to the Noctuidae family of the order Lepidoptera. Native to tropical and subtropical regions of America (Gui et al. 2022), it is mainly distributed in India, Laos, Myanmar, Thailand, Sri Lanka, Bangladesh, Vietnam, Australia and other countries (Kulye et al. 2021). The host plants of *S. frugiperda* exceed 350 species and primarily include major crops, such as maize, rice, millet, wheat, barley, soybean, sorghum, oats, and buckwheat (Gopalakrishnan and Kalia 2022). Guo et al. (2022) reported that *S. frugiperda* is a great threat to agricultural production and food security in China.

Currently, the use of chemical pesticides is the main method to prevent and control *S. frugiperda*. However, the excessive use of chemical pesticides not only causes

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toxicity in humans and animals but also pollutes the environment and causes public harm. In American countries, it has been reported that *S. frugiperda* has developed resistance to chemical pesticides with the gradual development of low to high resistance. At the same time, there are a large number of natural enemies in the natural environment of the *S. frugiperda*, parasitic natural enemies include *Telenomus remus*, *Trichogramma minutum*, *T. pretiosum*, *Microplitis rufiventris* and other parasitic wasps; predatory natural enemies mainly include Coccinellidae, Anthocoridae and *Podisus maculiventris* (Tepa-Yotto et al. 2021). Trichogramma parasitoids have been used extensively to control the eggs of *S. frugiperda* in America (Abbas et al. 2022). In terms of entomopathogenic microorganisms, they mainly include fungi, bacteria, viruses and nematodes. Multiple nuclear polyhedrosis viruses and baculoviruses are commonly used as a biological control agent to control of *S. frugiperda* (Haase et al. 2015). *Bacillus thuringiensis* has also been widely used for larval control of *S. frugiperda* (Fernanda et al. 2022). In terms of pathogenic fungi, mainly includes *Beauveria*, *Metarhizium, Paecilomyces*, *fumosorosea*, *Verticillium lecanii*, *Entomophthorales,* etc. The main entomopathogenic fungi (EPF) used to control *S. frugiperda* are *Beauveria*, *Metarhizium*, *Verticillium lecanii*, *Entomophthorales. B. bassiana* strain GHA, registered as BotaniGard® in the USA, has been commercialized for the control of second instar larvae of *S. frugiperda* (Wraight et al. 2010). A product of *Beauveria* have been registered in Africa for the control of young larvae of the pest (Lacey et al. 2015). Moreover, *Beauveria bassiana*, *Metarhizium anisopliae*, *Metarhizium rileyi*, and *Cordyceps fumosorosea* have been shown to be effective EPF against eggs and earlier instar larvae of *S. frugiperda* (Liu et al. 2022). However, there are few studies on other EPF for the control of *S. frugiperda*. Therefore, it is of great significance to search for new EPF to control the pest.

In this study, one fungus was found to infect *S. frugiperda* larvae in Xichang city, Sichuan Province, China. The aims of this study were to identify the pathogenicity of EPF to eggs and larval instars of *S. frugiperda*, which is important for the biological control of *S. frugiperda* and has potential for development of a new biological control agent.

Methods

Insect pest rearing

Spodoptera frugiperda individuals were collected from maize plants in Xichang County (102° E; 28° N), Sichuan Province, China, and maintained on maize leaves in an artificial climate chamber at 26 ± 1 °C with a relative humidity (RH) of 80 ± 5% and a 16 L:8 D photoperiod in a laboratory at the Institute of Entomology, Guizhou University, China.

Specimen collection and fungal isolation

In March 2020, one fungus-infected carcass of *S. frugiperda* was collected in Xichang, Sichuan Province, China, and the sample was transported to the laboratory in a 50-ml centrifuge tube. Under aseptic conditions, the body of the infected *S. frugiperda* carcass was cut into sections with a sterilized surgical blade; the tissue was immersed in 75% alcohol for 30 s, placed in a sterile Petri dish and rinsed three times with sterile water. The sterilized sections of the carcass were placed on sterilized PPDA plates and placed in an incubator with a constant temperature of 26 °C. Then, the strain was purified by the single-spore isolation method. To obtain a purified strain, spores were picked from the PPDA plate medium and placed in a 26 °C constant-temperature incubator for purification (Ghalehgolabbehbahani et al. 2022). The strain was maintained at the College of Agriculture, Sichuan Agricultural University.

Morphological identification

The spores of the isolated and purified strain were picked and inoculated on PPDA medium, 1 plate inoculated to 3 points, sterilized coverslips were inserted at 45° angles next to the inoculation points, and the plates were incubated in a constant-temperature incubator at 26 °C. When green spore mounds were observed with the naked eye, the inserts were removed, and the morphology of the conidia was observed under a light microscope. After 10–12 days, mature conidia were picked and photographed to observe and measure the size and shape of the spores. After approximately 14 days, the morphological characteristics of the colonies were observed and recorded (Faria et al. 2022).

DNA extraction, PCR amplification and nucleotide sequencing

The strain was identified by molecular analysis, and DNA was extracted using a DNA extraction kit (Beijing Adelaide Biological Co., Ltd.). The ITS, β-tubulin, and EF-1α sequences were then amplified by PCR using the primers shown in Table 1 (Bischoff et al. 2009). The amplification reaction volume was 10 μl and contained 1 μl of template DNA, 0.5 μl of upstream primer, 0.5 μl of downstream primer, 5 μl of 2 × Taq-Mix, and 3 μl of ddH_2O. The PCR amplification cycle was as follows: predenaturation at 95 °C for 30 s, denaturation at 95 °C for 30 s, and extension at 72 °C for 30 s, for a total of 34 cycles. The annealing temperatures for the amplification of the three sequences ITS, β-tubulin and EF-1α were 54 °C, 55 °C and 57 °C, respectively. The above PCR products were

Table 1 Primers of sequences for identifying fungal strains

Primer name	Primer sequences
ITS1	5′-TCCGTAGGTGAACCTGCGC-3′
ITS4	5′-TCCTCCGCTTATTGATATGC-3′
Pbeta-F	5′ a CCCTCCATTGTCTAGGACC- 3′
Pbeta-R	5′-CACATCATTGACGGGACTTAC-3′
983F	5′-GCYCCYGGHCAYCGTGAYTTYAT-3′
2218R	5′-ATGACACCRACRGCRACRGTYTG-3′

detected by 1% agarose gel electrophoresis, and the target bands were identified and sent to a company for sequencing (Shanghai Bioengineering Co., Ltd.).

Sequence alignment and phylogenetic analysis

The sequencing results of ITS, β-tubulin and EF-1α were compared with corresponding gene sequences in GenBank by Blast. The corresponding sequences of closely related species were downloaded to construct the phylogenetic tree, and the sequences of the fungi KY404947.1 and *Beauveria* MK804467.1 were used as out-groups. ClustalW and the neighbor-joining (NJ) method in MEGA-6 software were used to construct the tree, and the bootstrap value was set to 1000.

Bioassays

Preparation of the spore suspension of M. guizhouense Xct1

The strain obtained from the carcass was identified as *M. guizhouense* Xct1. The spores of strain Xct1 were cultured for approximately 2 weeks, scraped from the PPDA plates, placed in sterilized 0.1% Tween-80 solution, and shaken in a shaker for approximately 1 h to mix well. Then, using a hemocytometer plate, a master spore suspension solution with a concentration of 1.0×10^8 spores/ml was produced; this solution was then serially diluted to concentrations of 1.0×10^8, 1.0×10^7, 1.0×10^6, 1.0×10^5 and 1.0×10^4, 0.1% Tween-80 sterile water was used as controls (Cruz-Avalos et al. 2019).

Egg bioassays

Units of 100 freshly laid eggs attached to the oviposition paper were inoculated with the spore suspension by the immersion method for 10 s. 100 eggs per treatment, five treatments in total (1.0×10^8, 1.0×10^7, 1.0×10^6, 1.0×10^5 and 1.0×10^4 spores/ml), replicated 3 times. The control was treated with sterile water containing 0.1% Tween-80. Each unit was transferred to a Petri dish on wet filter paper. Eggs were observed continuously for 10 days. The hatching rates of the eggs in each concentration group were observed daily (Cruz-Avalos et al. 2019). During the egg hatching rate bioassay, eggs were observed for mycelium production, and all exposed samples were kept in an artificial climate chamber (26 ± 1 °C, RH 80% ± 5%, 16 L: 8 D).

Larvae bioassays

The pathogenicity of the different concentrations of *M. guizhouense* Xct1 spore suspensions to first–sixth larval instars of *S. frugiperda* was determined using the immersion method, with 0.1% Tween-80 sterile water as the control (Cruz-Avalos et al. 2019). For this bioassay, 30 larvae of first–sixth larval instars were selected, respectively, that were approximately the same size for each instar. Five total treatments, with 30 larvae per treatment, were replicated three times. The treatments were placed into a suspension of the tested spore concentration, larvae were removed after 10 s of exposure and then placed on filter paper to absorb excess liquid. The larvae were reared individually in a benzene permeable Drosophila tube with wet filter paper and fresh maize leaves for water and food, respectively, for 3–5 weeks. The larvae were continuously observed for 10 days, and the mortality rate of each larval instar group exposed to each concentration was recorded daily, dead larvae were surface-sterilized and transferred to sterilized Petri dishes lined with moistened tissue paper for incubation. All exposed samples were reared in an artificial climate chamber (26 ± 1 °C, RH 80% ± 5%, 16 L: 8 D). The larvae that died due to *M. guizhouense* Xct1 infection produced hyphae and green conidia, which were used as criteria for infection.

Pupae bioassays

Pathogenicity to pupae was determined by adding spores to the soil, and 1-d-old pupae of uniform size were selected for analysis (Pelo et al. 2020).

(1) Loamy soil was sieved through a 30-mesh sieve and then sterilized by dry heat in a constant-temperature oven at 160 °C for 120 min. Sterile water (121 °C, 0.1 mp, 40 min) was used as a moisture source when mixing the spores in the soil. Spore solution was individually added to achieve concentrations of approximately 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7 and 1.0×10^8 spores per gram of soil.
(2) The sterilized soil was poured onto a flat plate, the measured spore solution was added to the soil, and an appropriate amount of sterile water was added. The spore solution was stirred with a glass rod, mixed thoroughly with the soil and then placed into 500-ml containers. Each container contained approximately 300 g of the mixture.
(3) A total of five treatments and one control were established, with 30 pupae and 3 replications per

treatment. The containers were sealed with gauze, and the samples were kept in an artificial climate chamber (26 ± 1 °C, RH 80% ± 5%, 16 L: 8 D); the soil moisture content was maintained between 15 and 18% and was adjusted daily by calculating the change in the weight of the container. The mortality rate was recorded daily until adult emergence.

Statistical analysis

Data were recorded using Excel 2016 software, and the probit method in SPSS 27.0 was used to calculate LC_{50} values, plotting with Origin96.

Results

Morphological characteristics of the entomopathogenic fungi

Muscardine fungus-infected carcass of *S. frugiperda* was obtained from the field. The fungal strain was isolated and purified using the isolation method and named Xct1. The strain was cultured on PPDA plates, and the colonies were initially white and fluffy to flocculent, then turned green or yellow–green after sporulation. The conidia were single-celled, columnar or ellipsoidal, and transparent, with tapered ends, a smooth surface and a size of 6.8–9.7 μm × 2.1–3.3 μm. Under a light microscope, the hyphae were colorless, separated and branched. The ends of the conidiophores produced bottle-shaped metulae, from which strings of conidia were produced (Fig. 1). The characteristics were basically consistent with the morphology of the species *M. guizhouense*, described by Mayerhofer et al. (2015).

Sequencing and phylogenetic analysis

Three primer pairs, ITS1/ITS4, Pbeta-F/Pbeta-R and 983F/2218R of Xct1, were used (Fig. 2), and an ITS fragment approximately 600 bp in length, a tubulin fragment 1500 bp in length and an EF fragment 2000 bp in length were amplified from the genomic DNA of the Xct1 strain. Phylogenetic analysis by BLAST, incorporating the available gene sequences in GenBank (Figs. 3, 4 and 5). Most of the sequences with more than 90% similarity to the rDNA-ITS sequences of strain Xct1 are *M. guizhouense* and *M. robertsii*. In order to further clarify the *Metarhizium* between strain Xct1 and several *Metarhizium*, the rDNA-ITS sequences of *M. anisopliae*, *M. guizhouense*, *M. robertsii* and *M. carneum* published in GenBank were selected, respectively. The rDNA-ITS sequences of *M. anisopliae*, *M. robertsii*, *M. guizhouense* and *M. carneum* were selected for phylogenetic tree construction (Fig. 3). The results showed that the ITS sequence of Xct1 clustered on an evolutionary branch with that of an *M. guizhouense* strain, with 62% similarity.

Most of the sequences in GenBank with more than 90% similarity to the beta-tubulin sequence of strain Xct1 are *M. guizhouense* and *M. brunneum*, to further clarify the *Metarhizium* between strain Xct1 and several common *Metarhizium*. The sequences of *M. pinghaense*, *M. aureus* and *M. anisopliae* published in GenBank were used. The phylogenetic tree was constructed using the beta-tubulin sequences of *M. pinghaense*, *M. anisopliae*, *M. robertsii*, *M. guizhouense* and *M. brunneum* published in GenBank. The beta-tubulin sequences of strain Xct1 clustered on a small branch with that of an *M. guizhouense* strain, as shown in Fig. 4, with a similarity of 99%.

Most of the sequences in GenBank with 99% sequence similarity to EF-1α of strain Xct1 were *M. guizhouense* and *M. lepidiotae*, to further clarify the *Metarhizium* between strain Xct1 and several common *Metarhizium*, the EF-1α sequences of *M. acridum*, *M. majus*, *M. guizhouense* and *M. lepidiotae* published in GenBank were used. The phylogenetic tree was constructed using the EF-1α sequences of *M. acridum*, *M. majus*, *M. guizhouense* and *M. lepidiotae* published in GenBank. The analysis of the evolutionary tree in Fig. 5 shows that the EF-1α based sequence of strain Xct1 was the most similar to that of a *M. guizhouense* strain, with a similarity of 100%.

The phylogenetic tree results of the gene sequences ITS, β-tubulin and EF-1α strongly prove that strain Xct1 belongs to *M. guizhouense*; and considering these results combined with the morphological results of the strain, the pathogenic fungus Xct1, which can cause mortality in wild-type *S. frugiperda*, was finally identified as *M. guizhouense*.

Pathogenicity of fungal isolates against *S. frugiperda*

Larvae and pupae of *S. frugiperda* were exposed to spore suspensions of *M. guizhouense* Xct1 at different concentrations. As shown in Fig. 6, the pathogenicity of *M. guizhouense* Xct1 to the pupae of *S. frugiperda* was very poor, because the cumulative emergence rate of *S. frugiperda* exposed to all concentrations did not change significantly compared to that of *S. frugiperda* exposed to the control treatment after 1 day of inoculation. However, Xct1 showed different degrees of pathogenicity to different instar larvae of *S. frugiperda* exposed to Xct1 for 10 days. Figure 6 shows that with increasing time and spore concentrations, the cumulative mortality rates of larvae at different developmental stages presented an increasing trend. In the 1×10^4 spores/ml treatment group, the corrected mortality rates of the first–sixth larval instars of *S. frugiperda* were 32.56, 34.48, 22.09, 13.64, 10.11, and 4.55%, respectively, after the tenth day,. In the 1×10^8 spores/ml treatment group, the corrected mortality rates of the first–sixth larval instars of *S. frugiperda*

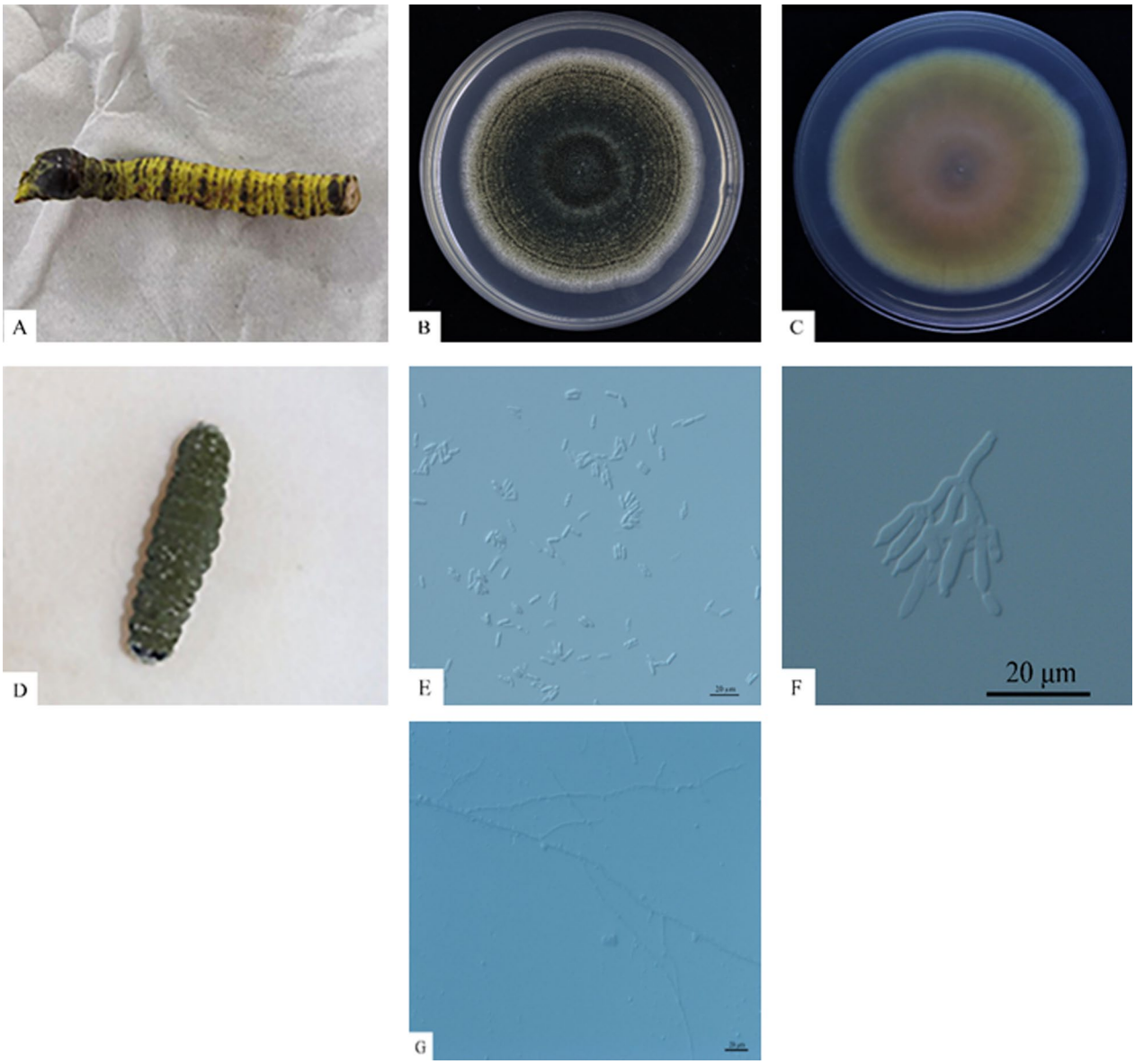

Fig. 1 Morphological characteristics of *Metarhizium guizhouense* Xct1. **A** Field worm samples; **B** Front of colony; **C** Back of colony; **D** Infected with fungus xct1 larvae; **E** Conidia; **F** Conidiophore; **G** hyphae

were 89.53, 86.21, 80.23, 72.73, 61.80, and 38.64%, respectively, after the tenth day, respectively. At a certain concentration, pathogenicity decreased with increasing developmental stage, while it increased with increasing spore concentration in the same instar group. The corrected mortality rates of the first, second, and third larval instars of *S. frugiperda* exposed to 1×10^8 spores/ml, 1×10^7 spores/ml and 1×10^6 spores/ml were more than 50%, and the corrected mortality rates of the fourth-instar larvae exposed to 1×10^8 spores/ml and 1×10^7 spores/ml exceeded 50%, at 72.73% and 54.44%, respectively. In the fifth instar larvae, after the tenth day, more than half of the larvae died after exposure to only 1×10^8 spores/ml, with a corrected mortality rate of 61.80%. The corrected mortality rate of sixth instar larvae did not exceed 50% at any of the five concentrations of treatment (Table 2).

As shown in Table 3, applying the probit model to calculate the LC_{50} values for *S. frugiperda* eggs and first–fifth—larval instars exposed to the Xct1 spore suspension. Since the final mortality rate of the sixth instar larvae did not exceed 50% on the tenth day, LC_{50} calculations were not performed for the sixth instar larvae. As shown in Table 3, the LC_{50} values for the eggs and first–fifth

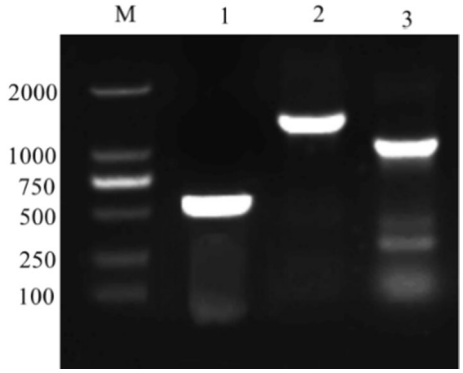

Fig. 2 Agarose gel electrophoresis of PCR products amplified from the ITS region of Xct1 isolates. *Note*: M: marker; 1: ITS sequence; 2: beta-tubulin sequence; 3: EF sequence

larval instars of *S. frugiperda* were 2.26×10^5, 8.12×10^4, 1.11×10^5, 5.55×10^5, 3.87×10^6, and 1.43×10^7 spores/ml, respectively, and the pathogenicity of *M. guizhouense* Xct1 to *S. frugiperda* gradually decreased with increasing developmental stage. The above results showed that strain Xct1 was more pathogenic to eggs and first–third larval instars than to fourth and fifth larval instars and was least pathogenic to sixth-instar larvae, which were the most difficult to infect and kill.

Discussion

Metarhizium contains many cryptic strains and its morphology varies on different culture media, the identification of strains by only their morphological characteristics is somewhat inadequate. The morphological characteristics of strain Xct1 were consistent with those of the *M. guizhouense* strain described by Mayerhofer et al. (2015). However, the minimum and maximum measurements of conidia in their study were slightly different from those in the present study. Nonetheless, the conidia size in the present study was basically consistent with that of Faria et al. (2022).

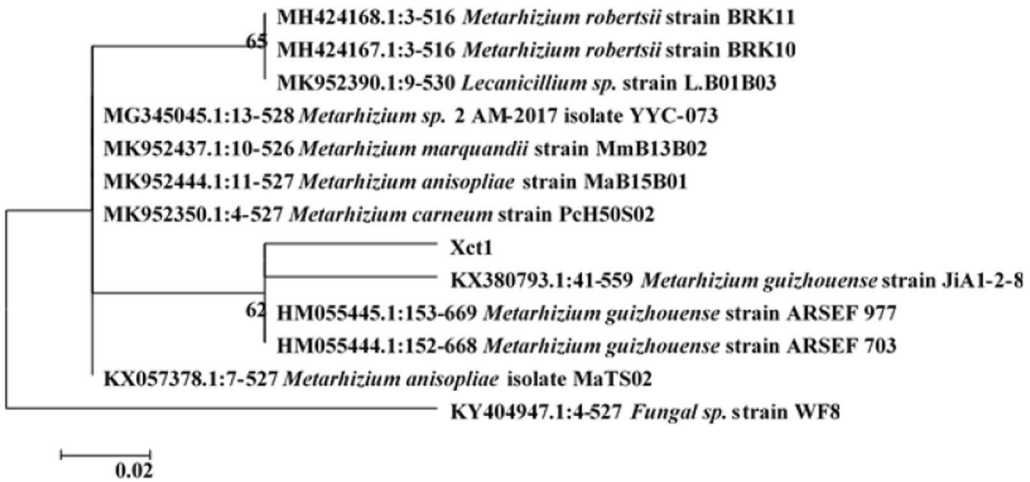

Fig. 3 Evolutionary tree of the Xct1 strain and *Metarhizium anisopliae* taxa based on rDNA-ITS sequences

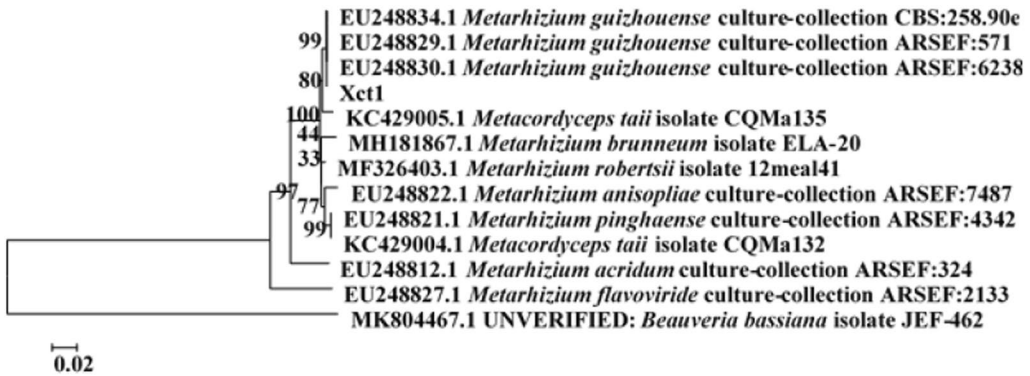

Fig. 4 Evolutionary tree of the Xct1 strain and *Metarhizium anisopliae* based on the beta-tubulin sequence

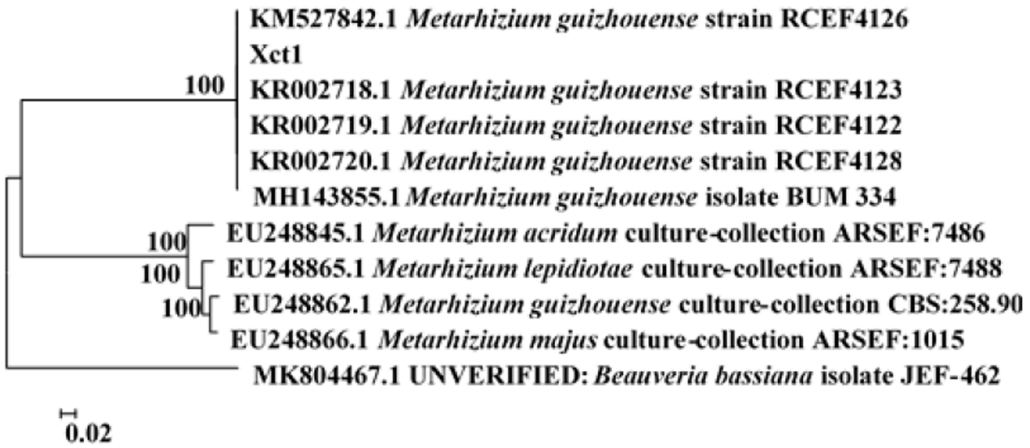

Fig. 5 Evolutionary tree of Xct1 strains and *Metarhizium anisopliae* taxa based on EF sequences

The classification system of *Metarhizium* proposed by Bischoff et al. (2009) is generally accepted by peer scholars as an important basis for strain identification; this system uses EF1-α, RPB1, RPB2 and β-tubulin multigene sequences combined with morphological characteristics to identify species of the strains. In this experiment, strain Xct1 was identified by the ITS1-4, β-tubulin and EF1-α sequences, among which the ITS1-4 sequence had the lowest similarity rate, at 62%. It is hypothesized that the function of ITS1-4 in strain Xct1 diverged during evolution, and the ITS1-4 sequence has a high variation frequency and magnitude, the main focus is on taxonomic efficiency compared to other genes. EF-1α sequences are highly effective in distinguishing cryptic strains of *Metarhizium* and analyzing relationships (Xie et al. 2021). The phylogenetic tree constructed from the EF-1α gene sequences showed that strain Xct1 clustered with *M. guizhouense*, with the highest similarity rate of 100%.

In this experiment, the pathogenicity of the strain to eggs, first–sixth larval instars and pupae of *S. frugiperda* was determined. The results showed that the strain was more pathogenic to eggs and larvae than to pupae. In a related study, pathogenicity to eggs was measured using 1×10^8 spores/ml concentration, and exposure to one of the strains of *M. anisopliae*, ICIPE 78, resulted in an 87% mortality rate in eggs (Akutse et al. 2019). In the present study, the corrected mortality rate was 90.81%, when egg pathogenicity at the same concentration was determined; this result was higher than the former, implying that this strain is potentially valuable in the control of eggs of *S. frugiperda*. After calculating the LC_{50} values for the first–fifth-instar larvae of *S. frugiperda* exposed to spore suspensions of *M. guizhouense* Xct1, it was found that the pathogenicity to larvae gradually decreased with increasing developmental stage; the LC_{50} values for the first–third-instar larvae were lower than those for the fourth–fifth larval instars. The result was similar to that of a previous study on the control of *S. frugiperda*, which reported that the spore solution was the most effective against first–third larval instars (Wang et al. 2022). It was reported that *M. anisopliae* at a concentration of 1×10^8 spores/ml had an LC_{50} value of 8.18×10^6 spores/ml in second instar larvae (García et al. 2011); while Barros et al. (2021) determined that the LC_{50} was 1.1×10^7 spores/ml; and in the present study, the LC_{50} was 1.11×10^5 spores/ml at the same instar larvae exposed to same concentration, indicating that it was more effective than those reported previously for second instar larvae. Some studies have determined the pathogenicity of *M. rileyi* to third instar *S. frugiperda* and found that the mortality rate reached 100% at a concentration of 1.0×10^8 spores/ml (Wang et al. 2022). The corrected mortality rate in third instar larvae, exposed to 1.0×10^8 spores/ml was only 80.23%. In addition, the Xct1 was very poorly pathogenic to the pupae of *S. frugiperda*, Visalakshi et al. (2020) found that *M. rileyi* FJMR2 was more than 90% pathogenic to the pupae, which greatly reduced the emergence rate of them. The reason for this difference in pathogenicity may be that the strains are different and its pathogenicity may differ, the cause may have multiple mechanisms, and the specific reasons need to be further investigated.

There are two main mechanisms for EPF infection of the host, one is mechanical pressure to penetrate the host's epidermis, such as the pressure generated by the directional growth of the germ tube during spore germination (Fang et al. 2009). The other is the degradation of the epidermis by enzymes, such as secondary metabolites, extracellular proteases, chitinase and esterase (Khan

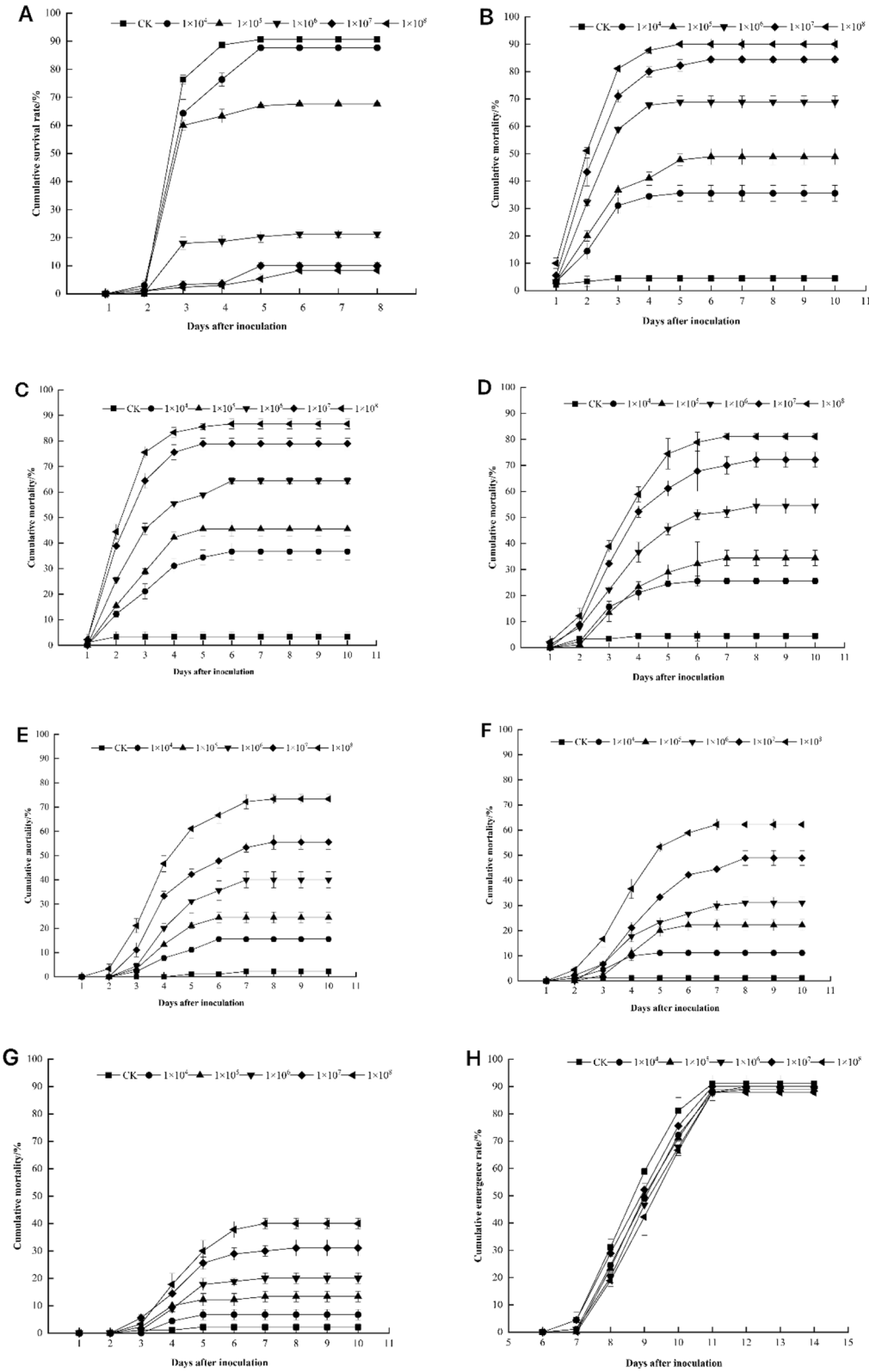

Fig. 6 Pathogenicity of *Metarhizium guizhouense* Xct1 to *Spodoptera frugiperda* larvae and prepupa. **A** egg; **B** first instar larva; **C** second instar larva; **D** third instar larva; **E** fourth instar larva; **F** fifth instar larva; **G** sixth instar larva; H: pupa

Table 2 Corrected mortality for 1–6 larval instars

Spore concentration (spores/ml)	Cumulative survival rate/%	Corrected mortality rate/% (instar larvae)					
	egg	First	Second	Third	Fourth	Fifth	Sixth
1×10^8	8.33Aa	89.53Aa	86.21Aa	80.23Aa	72.73Aa	61.80Aa	38.64Aa
1×10^7	10.00Aa	83.72Ab	78.16Ab	70.91Ab	54.44Bb	48.31Bb	29.55Ab
1×10^6	21.33Cc	67.44Bc	63.22Bc	52.33Bc	38.84Cc	30.34Cc	18.18Bc
1×10^5	67.67Dd	46.51Cd	43.68Cd	31.40Cd	22.73Dd	21.35Cd	11.36BCcd
1×10^4	87.67Dd	32.56De	34.48Ce	22.09Ce	13.64De	10.11De	4.55Dd

The different lowercase letters within the same column and the different uppercase letters within the same row indicate significant difference at 0.05 level ($p < 0.05$)

Table 3 Regression equation of pathogenicity of *Metarhizium guizhouense* Xct1 to *Spodoptera frugiperda* larvae

Stages	Pathogenicity regression equation	Correlation coefficient	LC_{50} (spores/ml)	95% confidence limits
egg	$y = -3.848 + 0.709x$	0.943	2.26×10^5	1.27×10^5–5.22×10^5
First	$y = -2.156 + 0.439x$	0.986	8.12×10^4	3.29×10^4–1.66×10^5
Second	$y = -1.910 + 0.383x$	0.980	1.11×10^5	4.10×10^4–2.43×10^5
Third	$y = -2.352 + 0.409x$	0.989	5.55×10^5	2.64×10^5–1.11×10^6
Fourth	$y = -2.716 + 0.412x$	0.990	3.87×10^6	1.93×10^6–8.68×10^6
Fifth	$y = -2.713 + 0.379x$	0.987	1.43×10^7	6.26×10^6–4.30×10^7
Sixth	–	–	–	–

LC_{50} represents the concentration required to kill 50% of the individuals in the insect population tested. "–" indicates that the final mortality rate of *S. frugiperda* larvae is less than 50%, and LC_{50} cannot be estimated

et al. 2016). Peng et al. (2021) found that during infestation, conidia of *M. rileyi* EFNL-8 were observed to adhere to the body surface of *S. frugiperda*, forming appressorium, invading the host as well as spreading mycelium in the body wall to grow and produce new conidia. We hypothesize that Xct1 infestation of *S. frugiperda* larvae mainly invades by means of germ tube or production of appressorium, followed by hypha growth and spore production on the surface of the body. However, the process of its invasion and its effect on *S. frugiperda* in vivo need further study.

Generally, several studies have been conducted to evaluate the pathogenicity of *B. bassiana*, *M. anisopliae*, *M. rileyi* and *M. robert* against *S. frugiperda*, however, there are few reports on the pathogenicity of *S. frugiperda* by *M. guizhouense*. In particular, the research that *M. guizhouense* can greatly reduce egg hatching rates, it provides an important basis to determine the best control period. The present study is an advance in this regard, meanwhile further research to show their activity under field conditions are needed.

Conclusions

In this study, the strain was identified as *M. guizhouense* by morphological identification and phylogenetic analysis, and named Xct1. Indoor biocontrol trials tested the pathogenicity of *S. frugiperda*, indicated that the effects of *M. guizhouense* Xct1 to *S. frugiperda* larvae gradually decreased with increasing the developmental stages. *M. guizhouense* Xct1 was more pathogenic to younger larvae of *S. frugiperda*. So, there is a possibility of using *M. guizhouense* as a new type of biopesticide. Further studies on their activity under field conditions are needed.

Abbreviations

S. frugiperda	*Spodoptera frugiperda*
M. guizhouense	*Metarhizium guizhouens*
M. anisopliae	*Metarhizium anisopliae*
T. pretiosum	*Trichogramma pretiosum*
EPF	Entomopathogenic fungi
M. rileyi	*Metarhizium rileyi*
B. bassiana	*Beauveria bassiana*
M. lepidiotae	*Metarhizium lepidiotae*
M. robertsii	*Metarhizium robertsii*
M. carneum	*Metarhizium carneum*
M. brunneum	*Metarhizium brunneum*
M. pinghaense	*Metarhizium pinghaense*
M. aureus	*Metarhizium pinghaense*
M. acridum	*Metarhizium acridum*
M. majus	*Metarhizium majus*

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Author contributions
JW and XW conceived the study, developed the methodology and wrote the article. LC, ZGNM, CCS, HNC, ZZ, JQ and CXJ performed laboratory tasks, collected and curated the data, and drafted preliminary reports. XW coordinated field surveys and performed the statistical analysis. All authors reviewed and approved the final manuscript.

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Declarations

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