Characterization of the Beauveria bassiana ga fungal strain isolated from the muscardine infected silkworms (Bombyx mori L.)

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Abstract. In this study, the focus was on isolating and characterizing the fungal strain Beauveria bassiana from muscardine-infected silkworms (Bombyx mori L.). The fungal strain isolated from the muscardine-infected silkworm, designated as GA, closely resembled Beauveria bassiana isolate SBI-Bb04 based on morphological analysis and comparison of the 18S rRNA nucleotide sequence with sequences in the NCBI database. Beauveria bassiana GA demonstrates the ability to grow on Sabouraud Dextrose Agar (SDA) medium. Optimal conditions for its growth were determined to be a pH range of 6-7 and a temperature range of 20-25°C. The composition of SDA, containing glucose and peptone sources, was found to be ideal for both growth and sporulation of this fungal strain. Furthermore, Beauveria bassiana GA exhibits the capacity to synthesize various extracellular enzymes, including chitinase, protease, cellulase, and amylase. These enzymatic activities contribute to its pathogenicity and suggest its potential as a biocontrol agent against insect pests.

1 Introduction

The silkworm (Bombyx mori L.) serves as a quintessential representative among Lepidopteran insects, playing a pivotal role in both agriculture and the economy by virtue of its utilization in the silk industry for natural fiber production. Nonetheless, the sericulture industry grapples with myriad challenges during the developmental process, ranging from climate change to pest infestations and diseases affecting silkworms. These factors collectively contribute to a substantial shortfall in actual cocoon yield compared to its potential. Notably, sick silkworms exhibit diminished cocoon yield, a consequence of the species' domestication over centuries, leading to a loss of inherent instincts to combat adverse conditions.

Silkworms are exceptionally vulnerable to various diseases instigated by diverse infectious agents, including fungi, bacteria, viruses, and protozoa, which severely impede cocoon production and pose a significant hindrance to sericulture development. Addressing this issue necessitates a shift towards prevention strategies over reactive control measures.

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following disease outbreaks. The prevalence of fungal diseases in silkworms ranks among the most widespread ailments, often attributed to parasitic fungi. These entomopathogens serve as biocontrol agents or "biostatagens" employed for managing insect pests.

In this context, the present study aims to isolate and characterize the fungal strain *Beauveria bassiana* from muscardine-infected silkworms (*Bombyx mori* L.). This endeavor holds promise for enhancing our understanding of disease management strategies in sericulture and may pave the way for innovative biocontrol approaches against insect pests.

## 2 Materials and methods

### 2.1 Material

The muscardine-infected silkworm cadavers were gathered from the Silkworm Disease Department at the Vietnam Sericultural Research Centre in Hanoi and subsequently transported to the laboratory. Upon arrival at the laboratory, the muscardine-infected silkworm cadavers were preserved at a temperature of 4°C in the refrigerator.

![Fig. 1. The muscardine infected silkworm cadavers.](image)

### 2.2 Methods

#### 2.2.1 Isolation of fungal strain

The isolation of fungi from the muscardine-infected silkworm cadavers followed the method outlined by Shanmugam et al. (2017). Firstly, the muscardine-infected silkworm cadavers were dissected into small pieces using a sterilized scalpel. Subsequently, they underwent surface sterilization with 70% ethanol for 15 seconds, followed by rinsing with sterile distilled water three times. The cadavers were then placed on sterile filter paper to dry under aseptic conditions. The surface-sterilized silkworm cadavers were transferred into Petri dishes containing Potato Dextrose Agar (PDA) supplemented with 40 μg/ml streptomycin to inhibit bacterial growth. All Petri dishes were then incubated in a controlled environment at 25 ± 2°C for a period of 7 to 14 days. The isolated fungal strains were subcultured multiple times to ensure purity. Each tested isolate, as well as the control, was replicated three times. The diameter of the fungal colonies was measured using a ruler, taking measurements at four points, and then calculating the average diameter, as described by Afifah et al. (2019).
2.2.2 Re-infection of the fungal strain in silkworm

This experiment evaluated the entomopathogenic of isolate strain in silkworms according to the method of Nguyen Van Hieu et al., 2022. The healthy Bombyx mori larvae (5th instar) were collected from the Vietnam Sericultural Research Centre – Hanoi, washed faeces và silks with sterile distilled water, boiled at three minutes, transferred into the erlenmeyer flasks and sterilized at 121°C for 15 minutes, then cooled to room temperature and inoculated the fungal spores (under in vitro condition). The control silkworms were dropped with sterile distilled water. There were three replicates as well as the control. After inoculating the fungal spores, the erlenmeyer flasks were cultured at room temperature to evaluate the ability of the fungus to grow on silkworms by observing changes in external morphology after 3 to 12 days.

2.2.3 Re-isolation of the fungal strain and identification of the fungal strain

After the silkworms exhibited the presence of white mycelium following re-infection, re-isolation of fungi was conducted. Using a sterile inoculating loop, fungal mycelium was extracted from the infected silkworms and transferred onto Potato Dextrose Agar (PDA) medium supplemented with 40 μg/ml streptomycin to inhibit bacterial growth. The fungal spores were harvested from the PDA medium after incubation for 7 to 14 days. Subsequently, the fungal morphology on the Petri dishes and the fungal spores were observed under a microscope to compare with the fungal strain initially isolated from the original silkworm cadavers. The isolated fungal strain was subcultured multiple times to ensure purity.

Following the re-isolation process, the fungal strain was sent for identification at DNA Sequencing Company Limited in Can Tho, Vietnam. This identification step aimed to determine the precise taxonomy and genetic characteristics of the fungal strain after re-infection in the silkworms.

2.2.4 The morphological characteristics of fungal strain

Based on the sequencing results, fungal colony morphology on PDA medium, and fungal spore morphology under a microscope to compare with the main taxonomic literature of Kendrick, and de Hoog (1972).

2.2.5 Effect of culture media on the growth of fungal strain

This study aimed to determine the effect of different culture media on the growth of fungal strain according to the method of Deb et al., (2017). For the selection of optimum culture media, the fungal strain was cultivated in the following five types of culture media such as...
SDA (Sabouraud Dextrose Agar, g/l: Glucose 40.0, peptone 10.0, yeast extract 2.0, agar 20.0, distilled water one liter, pH = 6.5 ± 0.2; sterilize at 121°C for 15 minutes), PDA (g/l): Potatoes, infusion from 200.0, dextrose 20.0, agar 15.0, distilled water one liter, pH = 5.6 ± 0.2; sterilize at 121°C for 15 minutes), PCA, CDA (Czapek-Dox Agar medium, g/l: Sucrose 30.0, NaNO₃ 2.0, K₂HPO₄ 3H₂O 1.0, MgSO₄.7H₂O 0.5, KCl 0.5, FeSO₄.7H₂O 0.01, agar 20.0, distilled water one liter, pH = 6.5 ± 0.2; sterilize at 121°C for 15 minutes), and Hansen medium amended with agar (g/l): Glucose 50.0, peptone 10.0, KH₂PO₄ 3.0, agar 20.0, distilled water one liter, pH = 6 ± 0.2; sterilize at 121°C for 15 minutes for 7 to 14 days. Use a sterilized cork borer to cut 5 mm diameter agar slices containing mycelia, then transfer to the center of the petri dishes for each media (one fungal slice per dish). A 5-mm-diameter mycelial plug taken from the growing plate of PDA culture medium at 25 ± 2°C was used for inoculation of each medium. All petri dishes were incubated in an incubation chamber for 7 to 14 days and three replications were maintained for each media. The growth rate of the colony was obtained by measuring its diameter after 7 to 14 days. The diameter was measured using a ruler by taking 4 points followed by calculating the average diameter (Afifah et al., 2019).

2.2.6 Effect of pH and incubation temperature on the growth of fungal strain

The physiological characteristics of new isolated B.bassiana GA strain were testing the ability to grow in medium with different pH, incubation temperature according to method described by Dhar et al., 2016.

Effect of pH

For the determination of optimum pH for fungal growth, the fungal strain B.bassiana GA was cultivated on the SDA medium with initial different pH such as 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0 as adjusted by adding either hydrochloric acid (0.1M HCl) or sodium hydroxide (0.1M NaOH). The sterilized medium was poured into petri dishes and allowed to solidify. Use a sterilized cork borer to cut 5 mm diameter agar slices containing mycelia, then transfer them to the center of the petri dishes for each pH (one fungal slice per dish). The dishes were incubated in an incubation chamber at 25 ± 2°C for 7 to 14 days and there were three replicates. The fungal colony diameter was recorded after 7 to 14 days. The diameter was measured using a ruler by taking 4 points followed by calculating the average diameter (Afifah et al., 2019).

Effect of temperature

In order to determine the effect of incubation temperature on growth of B.bassiana GA, the fungal strain was cultivated on SDA medium with pH 6 ± 0.2 in the following four different temperatures: 20°C, 25°C, 30°C, 35°C, and 40°C for 7 to 14 days. Mycelial growth was determined as in case of pH.

2.2.7 Effect of carbon and nitrogen sources on the growth of B.bassiana GA strain

The glucose in the SDA medium was replaced by four different carbon sources (sucrose, fructose, maltose, and lactose), and the peptone in the SDA medium was replaced by four different nitrogen sources (yeast extract, casein, KNO₃, and NH₄Cl). Use a sterilized cork borer to cut 5 mm diameter agar slices containing mycelia, then place them into the center of the petri dishes (one fungal slice per dish). The dishes were incubated in an incubation chamber at 25 ± 2°C for 7 to 14 days and there were three replicates. The basal media (SDA) was a control medium. The fungal colony diameter was recorded after 7 to 14 days. The diameter was measured using a ruler by taking 4 points followed by calculating the average diameter (Afifah et al., 2019).
2.2.8 The ability to synthesize extracellular enzymes

The enzyme assays were evaluated by adding the substrate of the enzymes into the media containing phosphate buffer with 2% agar: soluble starch 1% for amylase, chitin 1% for chitinase, carboxymethylcellulose 1% (CMC) for cellulase, and casein 1% for protease. The isolated strain was cultured in 100 ml of the PDB (Potato Dextrose Broth) liquid medium at 30°C for at least 7 days. Then, 1 ml of culture solution was centrifuged at 4°C at 10,000 rpm for 10 minutes to collect the supernatant solution. Use sterilized cork borer punch holes in agar to create a well on dishes, and use a micropipette to drop 100 µl of previously collected centrifugation solution into the agar well on the plate (a water-only control). The petri dishes were chilled at 4°C for 4 hours and then transferred to the incubation chamber at 30°C for 24 hours. After 12 hours, the culture dishes were flooded with Lugol’s iodine solution for chitinase, amylase, and cellulase; a solution of amido black 10B for protease. After 15 minutes, the dye was removed. The clear zone around the agar well revealed enzyme production after the dye discolor. The enzyme value > 1.0 indicates enzyme activity. The enzymatic activity index (EI) was calculated using the following formula (Bradner et al., 2019):

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\text{Enzymatic Index (EI)} = \frac{\text{Hydrolysis zone diameter}}{\text{Diameter of the agar well}}
\]

3 Results and Discussion

3.1 Isolation of the fungal strain

From the muscardine infected silkworm cadavers, a white fungal strain was isolated on the PDA medium was isolated. The mycelial growth reaches the maximum of 61.5 mm after 12 days of culture at 25°C ± 2°C. Infection of white muscardine in silkworm cadavers was characterized by symptoms like fungus with a colony of white hyphae growing on the integument of larvae. The isolated fungal strain appeared initially white and later turned yellowish; the texture was smooth like powdery, light spongy, and convex forming concentric circles; and light-yellow growth on the colony reverse side (Figure 2). Based on microscopic observation, the conidia are spherical or ellipsoidal, and non-septate. The mycelia are filamentous, branches with a septum.

According to description of general morphological characteristics of \textit{Beauveria bassiana} by Kendrick and de Hoog (1972), new isolated fungal strain in this study can belongs to \textit{Beauveria bassiana} fungus and named by Beauveria bassiana GA. This strain will reinfected silkworm and identification by comparing of 18S rRNA nucleotide sequences with known sequences on the NCBI database to get accurate conclusions.

Fig. 2. Colony (a), mycelial (b) and conidial morphology (c, d) of fungal strain \textit{Beauveria bassiana} GA on the PDA medium after 12 days.
3.2 Re-infection of the fungal strain in silkworm

The infection process with fungi occurs when the adherence of infective propagules to the host insect is followed by direct penetration through the cuticle. When conidia are attached to the body of insects, secretion of enzymes like proteases, and chitinases causes the degradation of cuticular components, allowing germination and growth of the fungus across the host surface. The fungus colonizes the cadaver and hyphae emerge and sporulate on the surface of the body of the insect. The insect was covered with a layer of white powder.

In this study, three days after spores’ inoculation, the mycelia are formed, and spores are germinated on the surface of silkworms. The fungal strain grew well on silkworm cadavers, and the fungus on hosts was floccose in appearance. The body of silkworms was covered with a layer of white powder. The fungal strain was cultured on five-week-old silkworms (Bombyx mori L.) and showed good growth after 7 days (Figure 3).

Fig. 3. Bombyx mori larvae (5th instar) after infection fungus.

After re-infection, from infected silkworms was isolated fungal strain with the morphology is similar to the fungal strain isolated from the muscardine infected silkworm cadavers (Figure 4).

Fig. 4. Colony (A), mycelial (B) and conidial morphology (C) of fungal strain Beauveria bassiana GA under the microscope after re-infection.

Powdery surface mycelium is velutinous to cottony, closely appressed to agar surface; the margin is white with colony interior white or changing to yellowish white to pale yellow. Conidia are spherical or broadly ellipsoid. The hyphae septate, are branched, and hyaline. The isolated fungal strain was sent to the DNA Sequencing Company Limited – Can Tho – Vietnam

3.3 Identification of the fungal strain

The 18S rRNA gene segment was sequenced using the Sanger method at DNA Sequencing Company Limited – Can Tho – Vietnam. The sequence was compared by BLAST on NCBI.
Based on the comparing of 18S rRNA nucleotide sequence of fungal strain *Beauveria bassiana* GA with the 18S rRNA nucleotide sequences of others fungal strains on the database of NCBI (fig. 4), the isolate fungal is the same clade as the *Beauveria bassiana*. So, the combination of morphological characteristics and molecular biology may be concluded isolated strain was previously determined to be *Beauveria bassiana* and named as *Beauveria bassiana* GA.

### 3.4 Effect of culture media on the growth of fungal strain

For the selection of optimum culture media, the fungal strain was cultivated in the following five types of culture media such as SDA, PDA, PCA, CDA, and Hansen medium for 7 to 14 days at 25°C ± 2°C. The results showed that the fungal colony on the SDA medium represented a higher growth rate than the colony on other media (Figure 6).

The isolated strain generated colony growth with different phenotypic characters in the different media. On the SDA medium, the surface of the fungal colony is white to creamy white powdery, spongy, and rough texture; light yellow growth on the reverse side of the colony. The colony surface of the fungal strain in most of the media had white to creamy white or later yellowish powdery. The morphological characteristic observed in the SDA medium was an undulated margin. On the CDA and Hansen medium, the growth form was
circular. On the PDA and PCA medium, the margin form was filamentous. The texture of the fungal strain was observed as smooth on the Hansen medium, followed by rough on the SDA, PDA, and PCA medium (Figure 7). On the 10th day, the highest growth was observed in the SDA medium. The colony diameter of the fungal strain reached a maximum of 57.5 mm on the SDA medium. Followed by the PDA medium was 48.5 mm, PCA medium was 38.5 mm, CDA medium was 34.5 mm. The least growth of fungal strain was observed in the Hansen medium, the colony diameter of the fungal strain reached a maximum of 27 mm (Figure 6). These results showed that SDA was the optimum culture media to grow well for the fungal strain.

The result is relatively consistent with the research of Deb et al., (2017) that the highest growth of *Beauveria bassiana* isolate was observed in SDA with the white colony color on both the front and yellow on the rear side, and the texture was rough. The results are also in line with Sharma et al., (2002) which reported that the good growth and sporulation of Beauveria sp. in SDB was due to the presence of peptone as a source of nitrogen. The composition of the SDA medium consisting of 40g of dextrose as carbon source and 10g of peptone as nitrogen source is comparatively higher than other selected media. The result is relatively consistent with the report of Senthamizhlselvan et al., (2010) that the significantly highest growth, sporulation, and biomass production of Beauveria spp. in SDA medium as compared to PDA medium.

### 3.5 Effect of pH on the growth of fungal strain

The medium pH also plays an important role in the growth, development, and metabolism of fungi (Dhar et al., 2016). After 12 days of incubation, the experiment results showed the growth of the *Beauveria bassiana* GA strain ranges from pH 4.0 to 10.0. Outside of this medium pH range, the growth of this strain was significantly retarded (Figures 8 and 9).

![Figure 8](image)

*Fig. 8.* The colony diameter of fungal strain *B. bassiana* GA in the different pH after 12 days (note: Bb: *B. bassiana* GA).
The fungal colony diameter reached maximum of 48.5 mm and 44.5 mm at pH 6 and pH 7. So the optimum pH for the good growth of the Bb strain was from about 6 to 7.

Fig. 9. The colony of Beauveria bassiana GA in the different pH on SDA medium after 12 days.

pH is an important abiotic factor that affects not only the survival of endoparasitic fungi but also their virulence towards insect pests. In the study, the fungal strain grew best on the SDA medium between pH 6 and 7. The result is relatively consistent with Karthikeyan et al., (2008) who reported that the pH 6-8 is considered the most suitable for growth and spore production of Beauveria bassiana. In addition, Ying and Feng (2006) studied the effect of pH level on the conidial production of Beauveria bassiana in Sabouraud’s dextrose medium and reported that the culture conditions were optimal at pH 5.0-6.0. Moreover, an optimum pH of 6 - 7 of SDA medium which is found to be best for the growth and biomass production of Beauveria bassiana.

3.6 Effect of temperature on the growth of fungal strain

Temperature is a factor that directly affects germination, sporulation, and mycelial growth (Dhar et al., 2016). The experiment evaluated the effect of different temperatures on the growth of the fungal strain on the SDA medium at different temperature. The results showed that the fungi may grow from about 20°C to 25°C, (Figure 10). At 25°C, the fungal colony diameter reached maximum of 76.5 mm after 14 days. At 20°C, the fungal colony diameter reached maximum of 67 mm after 14 days. Followed by the fungal colony diameter reaching a maximum of 46.5 mm at 30°C. Outside of this temperature range, the B.bassiana GA strain grew slowly (at 35°C) or did not grow (at 40°C). The morphology of the fungal strain is described in Figure 11.

Fig. 10. The colony diameter of Beauveria bassiana GA fungal strain in the different temperatures after 14 days.
Temperature can affect insect pathogens in a variety of ways such as influencing the germination, growth, and viability of fungi in the host insect and the environment. High temperatures may inactivate insect pathogens before contact with the pest insects or may reduce or accelerate the development process inside the insect depending on the temperature of the insect pathogen and the host insect. Conversely, low temperatures may reduce or stop germination and growth, thereby weakening or prolonging successful infection (Zimmermann, 2007). For the isolated strain, from infected silkworms in tropical climates, based on experiments cultivation of fungal strain at different temperatures, the results showed that the growth rate of fungal mycelium was best at 25°C. The results are relatively consistent with the research of many authors; according to Muller Kogler (1965) and Campbell (1977), in *Beauveria bassiana*, the optimum temperature is 23-28°C, the minimum 5-10°C, and the maximum about 30-38°C, depending on the different climates of the isolates tested; according to Roberts and Campbell (1977) and Fargues et al., (1992), the *Beauveria bassiana* is mesophilic, the optimal temperature for most Bb isolates is between 20 and 30°C. According to Tefera & Pringle (2003), among African *Beauveria bassiana* isolates, germination, radial growth, and sporulation of all isolates were retarded at 15-35°C, while the optimum temperature of different isolates was between 20 and 30°C or 25-30°C (Ekesi et al. 1999) because Africa is a hot region so isolates grow slowly. So that, some isolates have a wide range of optimal temperatures while others are much more restricted depending on sampling places.

### 3.7 Effect of nutritional conditions on the growth of fungal strain

The fungal strain requires a carbon source for conidial swelling and germ tube formation and a nitrogen source for hyphal development (Boucias and Pendland, 1998). Carbon and nitrogen are the most vital nutrients required for growth and sporulation of fungi (Campbell et al., 1983).

#### 3.7.1 Carbon source

In this experiment, the fungal strain was well grown in all different carbon sources tested. Carbon sources (sucrose, fructose, maltose, and lactose) grew nearly as well as the control (glucose) on basal media (SDA) (Figure 12).
The results showed that the highest growth of fungal strain was observed in the basal media with carbon source glucose after 12 days. Besides, all the tested carbon sources supported the growth and development of the fungal strain. The growth diameter is shown in Figure 13.

**Fig. 13.** The colony diameter of *Beauveria bassiana* GA fungal colony in the different carbon sources.

### 3.7.2 Nitrogen source

Through the experiment, the results showed that the highest growth of fungal strain was observed in the basal media with nitrogen source is peptone after 12 days (Figure 13). The growth diameter is shown in Figure 14.

**Fig. 14.** The *Beauveria bassiana* GA fungal colony in the different nitrogen sources after 12 days.

The colony fungal in the medium has peptone showed the highest amount among the remaining different media. In the medium containing inorganic nitrogen sources, fungal mycelium grew slowly or did not grow.

**Fig. 15.** The colony diameter *Beauveria bassiana* GA fungal strain in the different nitrogen sources.
The most important nutritional components in the culture medium that affect biomass yield and metabolite production are carbon and nitrogen. The *Beauveria bassiana* strain requires a carbon source for spore swelling and germ tube formation and a nitrogen source for mycelial growth (Boucias and Pendland, 1998). Carbon and nitrogen are the most important nutrients required for fungal growth and sporulation (Campbell et al., 1983). In the study, *B. bassiana* GA strain may be cultured on different media containing different carbon sources such as glucose, sucrose, fructose, maltose, and lactose; and different nitrogen sources such as peptone, yeast extract, casein, KNO₃, and NH₄Cl. The results indicate that four carbon sources that were replaced in the SDA medium can still be used to culture the *Beauveria bassiana* GA strain. The growth of the *B. bassiana* GA strain was reduced by the presence of potassium nitrate (KNO₃) and NH₄Cl as nitrogen sources, irrespective of the carbon source. The medium which contains carbon (glucose) and nitrogen (peptone) sources, was the most optimum medium to produce high biomass in fungal strain. The results are also consistent with Sharma et al., (2002) which reported that the good growth and sporulation of *Beauveria* in SDB was due to the presence of peptone as a source of nitrogen. So the combination of glucose with peptone in the basal media is the optimum component for the good growth of the *B. bassiana* GA strain.

3.8 The ability to synthesize extracellular enzymes of *B. bassiana* GA fungal strain

The mode of infection of entomopathogenic fungi such as *Beauveria bassiana* involves spore deposition on the insect cuticle followed by the germ tube formation, which through enzymatic and mechanical action penetrates the epidermis layer (Hajek and Leger, 1994). The results of the study indicate that the fungal strain exhibits the capability of producing various extracellular enzymes (Figure 16). The enzyme activity index, as depicted in Figure 20, reveals that the *B. bassiana* GA strain displays the highest chitinase activity index, reaching 3.9. Chitinases, along with proteolytic and hydrolytic enzymes, play crucial roles in the pathogenicity of insect-parasitic fungi (Samuels & Paterson, 1995; Charnley & Leger, 1991; Nguyen Van Hieu et al., 2020). Additionally, the fungal strain exhibits amylase and cellulase activity. These assay results indicate that the fungal strain is proficient in producing several extracellular enzymes, including chitinase, protease, and amylase.

![Fig. 16. The ability to synthesize extracellular enzymes of B.bassiana GA.](image)

In conclusion, the study suggests that the use of Sabouraud Dextrose Agar (SDA) medium, along with an optimum temperature range of 20-25°C and pH range of 6-7, promotes maximum growth and sporulation of the isolated strain of *Beauveria bassiana* GA.
Conclusion

From the muscardine-infected silkworm (*Bombyx mori* L.), a fungal strain named GA was isolated. After re-infecting silkworms with this fungal strain, a similar fungal strain was isolated from the diseased silkworms, exhibiting characteristics akin to the infectious strain GA. Through morphological analysis and comparison of the 18S rRNA nucleotide sequence with sequences in the NCBI database, it was determined that fungal strain GA bears close resemblance to *Beauveria bassiana* isolate SBI-Bb04. Consequently, it was designated as *Beauveria bassiana* GA.

*Beauveria bassiana* GA demonstrates robust growth on Sabouraud Dextrose Agar (SDA) medium. The optimum pH and temperature for its maximal growth were identified as 6-7 and 20-25°C, respectively. The composition of SDA, which includes glucose and peptone sources, proves to be the ideal medium for both growth and sporulation of this fungal strain. Notably, *Beauveria bassiana* GA exhibits the capacity to synthesize various extracellular enzymes, including chitinase, protease, cellulase, and amylase. These enzymatic activities contribute to its pathogenicity and suggest its potential as a biocontrol agent against insect pests.

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