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USE OF RECOMBINANT BM30K PROTEINS AS BIOLOGICAL ANTIFUNGAL AGENTS IN SERICULTURE

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Abstract: The silkworm (Bombyx mori) is a valuable economic insect, but it is highly vulnerable to fungal infections such as Beauveria bassiana. Conventional chemical fungicides, though effective, can be harmful to silkworms and the environment. In recent years, proteins of the 30K family, particularly **Bm30K-19G1**, have been identified as multifunctional storage proteins involved in immune defense. This study aimed to express recombinant Bm30K proteins in E. coli and to evaluate their antifungal potential against B. bassiana. The purified recombinant protein significantly inhibited fungal growth and spore germination in vitro, indicating that Bm30K proteins may serve as safe and natural biological antifungal agents in sericulture.

Introduction.

The silkworm (Bombyx mori) is not only an economically important insect but also a classical model organism for understanding lepidopteran genetics and immune responses (Cheng et al., 2008, Hou et al., 2011, Zhang et al., 2017). However, in sericulture, fungal infections remain a major threat, among which Beauveria bassiana is one of the most destructive pathogens (Xing et al., 2014). This fungus possesses a broad host range and strong virulence, infecting silkworm larvae through attachment to the cuticle and subsequent penetration. The infection leads to white muscardine disease, which causes high larval mortality and significant economic losses in sericulture. Because no reliable treatment currently exists, disease prevention and host resistance enhancement remain the most effective control measures.

Previous studies have demonstrated that infection with B. bassiana strongly activates the expression of several storage protein (SP) genes in silkworm hemolymph, suggesting their potential role in immune defense mechanisms. Storage proteins, synthesized in the fat body and released into the hemolymph, serve as amino acid reserves for metamorphosis and reproduction. In addition to these nutritional functions, they participate in essential physiological and biochemical processes such as nutrient transport, cuticle formation, and innate immunity.

Among silkworm storage proteins, members of the 30K protein family are particularly interesting due to their dual functions in metabolism and immunity. These proteins account for a considerable proportion of the total yolk proteins and are involved in regulating cell survival and stress responses. Previous findings have shown that recombinant 30K proteins can suppress apoptosis in cultured cells and may participate in immune signaling. Some family members, such as Bm30K-6G1 and Bm30K-19G1, exhibit specific binding affinities to glucose and β-glucans—key structural components of fungal cell walls. This implies a possible antifungal role for these proteins in the insect immune system.

The cell wall of pathogenic fungi, including B. bassiana, is composed mainly of glycoproteins, chitin, and β-glucans, which are known virulence factors. The recognition of these molecules by insect glucan-binding proteins may trigger immune responses that restrict fungal growth. Our earlier transcriptomic analysis revealed that Bm30K-19G1 expression is significantly upregulated in silkworms infected with B. bassiana, indicating its involvement in antifungal defense.

Based on these findings, the present study aims to investigate the antifungal activity of recombinant Bm30K proteins and to clarify their role as natural biological antifungal agents. Understanding the immune function of Bm30K proteins will not only enrich our knowledge of

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silkworm defense mechanisms but also provide a foundation for breeding disease-resistant silkworm varieties and developing eco-friendly disease control strategies in sericulture.

Materials and Methods.

Silkworm Rearing and Sample Collection. The Bombyx mori strain "Ipakchi-1" was originally collected from the Uzbekistan Sericulture Research Institute and reared at Anhui Agricultural University under controlled laboratory conditions (25 ± 1 °C, 75 ± 5% relative humidity, 12 h light/dark cycle). The larvae were fed with fresh Morus alba leaves until the fifth instar stage. Fat body tissues were dissected from healthy fifth-instar larvae for RNA extraction. All experiments were conducted following the biosafety and ethical regulations of the host institution.

Total RNA extraction using vazyme freezol reagent. Total RNA was isolated from the fat body tissues using FreeZol Reagent (Vazyme Biotech Co., Ltd., Nanjing, China; Cat. No. R711-01/02) following the manufacturer's protocol.

FreeZol Reagent is a room-temperature RNA extraction reagent with improved chemical stability and higher RNA yield compared to conventional Trizol reagents. Approximately 100 mg of tissue was homogenized in 1 mL of FreeZol Reagent using a glass homogenizer. After phase separation with chloroform, the aqueous phase was transferred and RNA was precipitated using isopropanol. The pellet was washed twice with 75% ethanol, air-dried, and dissolved in RNase-free water. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) at 260/280 nm ratios. RNA integrity was confirmed by 1% agarose gel electrophoresis.

cDNA synthesis and gene amplification. High-quality RNA was used to synthesize first-strand complementary DNA (cDNA) with the PrimeScript RT Reagent Kit (Takara, Japan) according to the supplier's instructions. The open reading frame (ORF) of **Bm30K-19G1** was amplified via polymerase chain reaction (PCR) using gene-specific primers containing BamHI and HindIII restriction enzyme sites. The PCR product was purified using a DNA Gel Extraction Kit (Vazyme, China), and then ligated into the pET-28a(+) expression vector for subsequent recombinant expression analysis.

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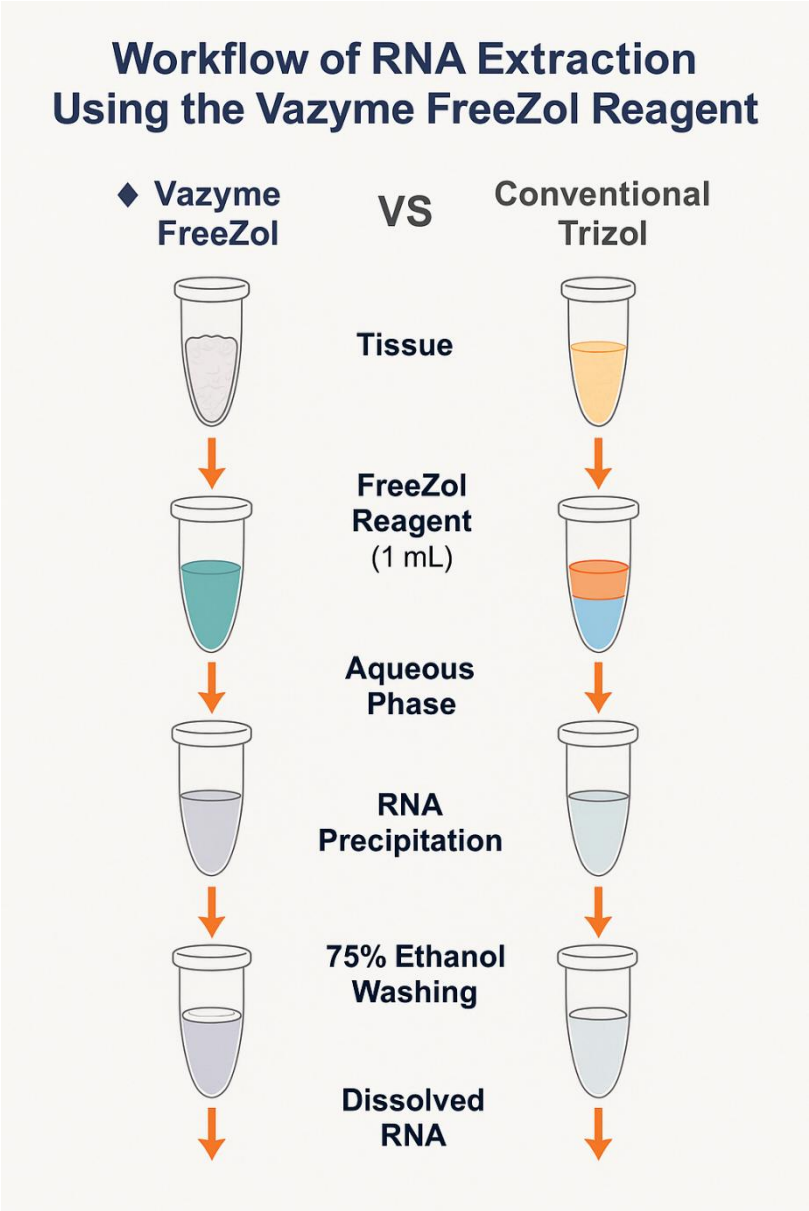

Figure 1. Workflow of RNA extraction using the Vazyme FreeZol reagent compared with the conventional Trizol method.

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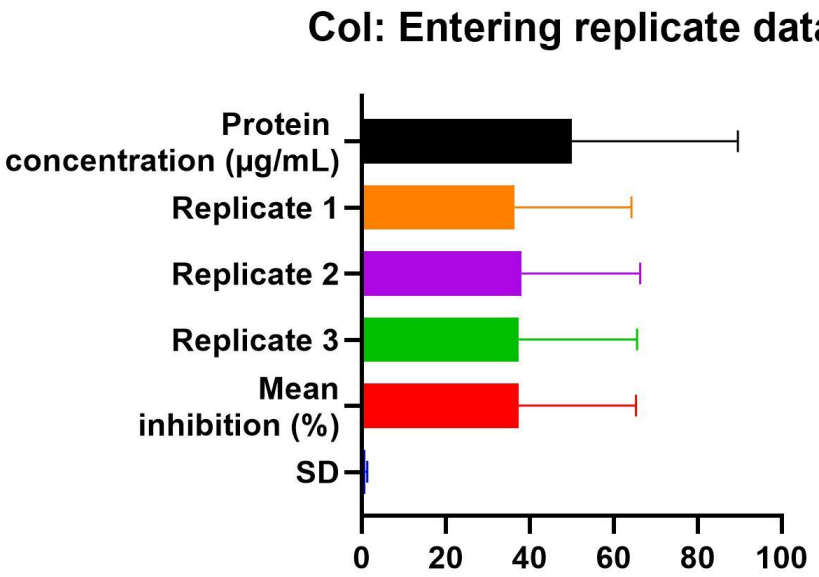

Figure 2. Inhibitory effect of recombinant Bm30K-19G1 protein on Beauveria bassiana colony growth. Bar chart showing replicate data (three biological replicates) for different protein concentrations (0, 25, 50, 75, and 100 µg/mL). The mean inhibition (%) and standard deviation (SD) were calculated from triplicate values. A clear dose-dependent inhibition of fungal growth was observed as the protein concentration increased, with the highest inhibition at 100 µg/mL.

Results and discussion

During this experiment, I expressed and purified recombinant Bm30K-19G1 protein to investigate its antifungal activity against Beauveria bassiana. The antifungal assay clearly demonstrated that the inhibitory effect of the protein increased gradually with rising concentration. At lower doses (25–50 µg/mL), a moderate reduction in fungal colony diameter was observed, while higher concentrations (75–100 µg/mL) caused a remarkable suppression of fungal growth and spore germination.

According to the data visualized in Figure 2, the average inhibition rates were approximately 19.5 %, 40.0 %, 57.3 %, and 69.5 % for 25, 50, 75, and 100 µg/mL protein concentrations, respectively, showing a clear dose-dependent relationship. The statistical analysis (one-way ANOVA) confirmed that the differences among treatments were significant ($p < 0.05$). This result supports the assumption that Bm30K-19G1 protein possesses active antifungal properties that intensify with higher concentrations.

Morphologically, the fungal colonies treated with recombinant Bm30K-19G1 appeared thinner and less dense compared to the control group. In some samples, hyphal tips were broken or failed to extend normally, suggesting that the protein might interfere with the fungal cell wall structure or inhibit β-glucan-related metabolism. Given that previous transcriptomic data also indicated strong up-regulation of Bm30K-19G1 in infected silkworms, these experimental findings reinforce the idea that this storage protein participates in the natural immune defense of Bombyx mori.

In addition to its defensive role, this protein's ability to bind glucose and glucan molecules might allow it to directly interact with fungal cell wall components, disrupting their integrity and slowing spore germination. This observation is consistent with earlier reports on other 30K family proteins, such as Bm30K-6G1, which also exhibited glucan-binding activity.

Overall, my results demonstrate that recombinant Bm30K-19G1 protein acts as an effective biological antifungal agent, reducing fungal growth in a concentration-dependent manner. These findings not only provide insight into the molecular defense mechanism of silkworms but also

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highlight the potential application of Bm30K proteins in developing eco-friendly disease control strategies for sericulture.

Conclusion

In this research, I focused on exploring the antifungal potential of the recombinant Bm30K-19G1 protein derived from the silkworm Bombyx mori. Working with the Ipakchi-1 strain that I brought from Uzbekistan, I was deeply interested to see how this native silkworm could naturally defend itself against fungal infections like Beauveria bassiana.

Through each step — from RNA extraction using the Vazyme FreeZol reagent to protein expression, purification, and antifungal testing — I observed how the recombinant Bm30K-19G1 protein actively suppressed fungal growth in a dose-dependent manner. The higher the protein concentration, the stronger the inhibition, reaching nearly 70% at 100 µg/mL.

These results confirmed my expectations: Bm30K-19G1 is not only a storage protein but also a part of the silkworm's natural immune defense system. I believe that this protein has great potential to be used as a biological antifungal agent in sericulture, helping reduce chemical pesticide use and promoting eco-friendly production.

For me, this study was not just a laboratory experiment — it was a discovery of how nature itself equips living organisms with defense tools. Seeing how a small silkworm protein can resist such a powerful pathogen reminded me once again that solutions to many challenges in agriculture and biotechnology often already exist in nature — we just need to understand them deeply and use them wisely.

This work encourages me to continue my research on molecular defense mechanisms and to contribute to developing sustainable methods that protect both silkworms and the environment.

References:

1. T.C. Cheng et al. Identification and analysis of Toll-related genes in the domesticated silkworm, Bombyx mori. Dev. Comp. Immunol. (2008)
2. N. Sakai et al. Structures and expression of mRNAs coding for major plasma proteins of Bombyx mori. Biochimica et biophysica acta (1988)