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Molecular Diagnostics Methods for the Identification of Diseases of Silkworm

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Silkworms (*Bombyx mori* and non-mulberry species) are economically important insects for silk production but are highly vulnerable to diseases caused by bacteria, viruses, fungi, and microsporidia. These infections lead to significant economic losses due to the lack of effective treatments and the limitations of conventional diagnostic methods, such as microscopy and symptom-based observation, which are often time-consuming and less accurate. Molecular diagnostic techniques—including PCR, multiplex PCR, reverse transcription PCR, quantitative PCR, loop-mediated isothermal amplification (LAMP), ELISA, fluorescent antibody assays, and nucleic acid-based methods like Southern and Northern blotting—offer rapid, sensitive, and specific detection of silkworm pathogens. The integration of bioinformatics tools enables precise primer and probe design, further improving assay accuracy. These advanced molecular approaches allow early pathogen identification, better disease management, and sustainable silk production, making them essential tools for modern sericulture.

Keywords: Silkworm, Sericulture, Molecular diagnostics, PCR, LAMP, ELISA, Pathogen detection, Disease management

Introduction

India secures the second largest producer and consumer of silk in the world. Silk is a lustrous natural fibre from the insect which is utilised for its proper ties of elastic nature, skin affinity, thermal insulation and tensile strength. The mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), and non-mulberry silkworms (*Antheraea* sp. and *Samia* sp.) of the Saturniidae family are the economic insects utilised for commercial silk production. Silkworms are more prone to many diseases caused by bacteria, viruses, fungi and parasites, which ultimately affect the silk production. These diseases, which are primarily caused by microorganisms such as bacteria, fungi, viruses, and microsporidia, frequently result in significant financial losses because there are no effective treatments and general preventive measures are insufficient (Sivaprasad *et al.*, 2021). Traditional methods of pathogen identification, such as microscopy and laboratory culturing, are limited by low accuracy and low efficiency. Further, these conventional diagnostic methods were based on post-infection symptoms such as pepper- like spots (Nosema infection), swelling of the intersegmental region (nucleopolyhedrovirus (NPV)) and oily specks on integument caused by fungal infection. Therefore, creating efficient management plans and guaranteeing sustainable silk production requires a thorough grasp of the etiology, epidemiology, and pathogenesis of these diseases (Sisodia *et al.*, 2019). To overcome these limitations, molecular diagnostics methods were used for early identification of the diseases. Molecular diagnostics involves the use of

DNA/RNA or protein- based molecular biology techniques to identify and diagnose infectious agents. Bioinformatics tools can also be used to identify the pathogen genomes, which in turn helps in understanding the pathogen's genetic make-up and identifying potential target sites for molecular diagnostic assays. Primers and probes specific to the target pathogen can be designed with the use of bioinformatics tools.

Molecular tools for the detection of silkworm pathogens

Polymerase chain reaction

A thermostable DNA polymerase enzyme copies the target DNA in PCR in the presence of nucleotides and specific primers. The target DNA is exponentially amplified by repeated heating and cooling cycles in a thermocycler, which results in rounds of target DNA denaturation, primer hybridisation and primer extension (Clark et al., 2019). PCR has been extensively used to detect different pathogens such as viruses, bacteria, fungi and microsporidia pathogens in silkworms. For example, the microsporidian pathogen of the Muga silkworm, A. assamensis, was identified as Nosema sp., which involved a PCR-based assay of the SSU- RNA gene and its sequence analysis (Subrahmanyam *et al*., 2019).

Multiplex PCR

Multiplex PCR (M- PCR) enables the simultaneous detection of several target sequences by incorporating multiple sets of primers, thus enabling the detection of multiple target genes in a single step. Simultaneous detection of Nosema bombycis, NPV, and densonucleovirus is possible with a single tube reaction as reported by Ravikumar *et al*. (2011).

Reverse Transcription PCR

Reverse transcription PCR (RT- PCR) is particularly useful for the detection of RNA viruses of silkworms. In RT- PCR, the viral genomic RNA is first converted into cDNA using a reverse transcriptase enzyme. The cDNA is then amplified using PCR, which involves the selective amplification of a specific DNA sequence using primers (short stretches of DNA that are complementary to the target sequence).

Quantitative PCR

This technique is useful for determining the relative or absolute abundance of a target DNA or RNA sequence in a sample. The technique was found to be 100 times more sensitive than conventional PCR for diagnosing Nosema sp. The detection limit of qPCR was found to be 100 pg of spore DNA, whereas the conventional PCR detection limit was 10 ng of spore DNA (Esvaran *et al*., 2019).

Loop-mediated Isothermal Amplification

LAMP is a rapid and sensitive method for the amplification of DNA or RNA that can be performed at a constant temperature, typically around 65–68°C. This technique has several advantages over PCR, including the ability to amplify DNA or RNA at a single temperature, the ability to detect amplification in real time, and the ability to amplify DNA or RNA in the presence of high levels of contaminants (Deepika *et al*., 2024).

Enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is the most common immunoassay, in which antigen-antibody complexes are absorbed into wells in plastic microtitre plates. This method is suitable for large sample numbers and can detect low concentration of the virus. ELISA has been proven to be a sensitive and reliable method for the detection of grasserie disease. With ELISA, viruses can be detected within 1 day after inoculation, whereas with light microscopes, the virus can be detected after 3 days post-inoculation (Deepika et al., 2024)

Fluorescent antibody technique

The fluorescent antibody technique employed was first developed by Coons and Kaplan in 1950, and has been successfully applied in clinical diagnostic work involving bacteria, fungi, protozoa, rickettsiae and viruses. Krywienczyk (1963) found that the fluorescence in the cytoplasm of infected cells before crystallisation of polyhedral, which was said that the inclusion body proteins are synthesised in the cytoplasm.

Nucleic acid-based method

Southern blotting is the transfer of DNA fragments from an electrophoresis gel to a membrane support. The transfer or a subsequen treatment results in immobilisation of the DNA fragments, so the membrane carries a semipermanent reproduction of the banding pattern of the gel. After immobilization, the DNA can be subjected to hybridization analysis, enabling bands with sequence similarity to a labelled probe to be identified. The most popular membranes are made of nitrocellulose, uncharged nylon, or positively charged nylon. Northern blotting involves the use of electrophoresis to separate RNA samples by size and detection with a hybridisation probe complementary to part of or the entire target sequence. It refers specifically to the capillary transfer of RNA from the electrophoresis gel to the blotting membrane (Clark et al., 2019).

Conclusion

The diseases in sericulture are a major hurdle to gain profit. Molecular diagnostics has become an essential tool in the field of sericulture for the detection and identification of silkworm pathogens. Sericulture has been transformed by the use of molecular techniques for pathogen identification, which offer a major advantage over conventional methods by enabling the quick and precise detection of disease-causing agents.

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