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Molecular Approaches for Detection of Pebrine Disease in Sericulture

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ABSTRACT

Nosema bombycis is an obligate intracellular parasite that causes pebrine disease by infecting Bombyx mori. The disease is transmitted transovarially and spreads rapidly in silkworm seed multiplication farms. Pebrine is the only quarantine requirement in silkworm egg production and is usually monitored by mother insect examination and light microscopy for specific spores. Practically spore detection is a bit technical, laborious and the life cycle stages of N. bombycis can also be observed by microscopy. However, routine monitoring of pebrine disease through light microscopy is limited by specificity and sensitivity. More practical and feasible diagnostic methods for pebrine monitoring have emerged with the development of immunodiagnostic and nucleic acid based detection techniques. Immunodiagnostic assays such as latex agglutination and ELISA using polyclonal and monoclonal antibodies were developed for diagnosis and characterization of microsporidian infections. However, false positives and low-level infections made immunoassays less reliable. Nucleic acid-based detection methods using DNA sequences specific for N. bombycis can detect particularly low levels of infection. Major DNA diagnostics for detecting N. bombycis include PCR, loop-mediated isothermal amplification (LAMP) and lateral flow assays that are high-throughput and quantitative, as well as capable of detecting multiple diseases simultaneously. Molecular diagnostic techniques are more advantageous than microscopy because of increased sensitivity, specificity, rapid and easy result interpretations. The molecular diagnostic tests described for the identification of microsporidia infecting mulberry silkworm are given in this chapter.

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KEYWORDS: *Nosema bombycis, pebrine, diagnostic, detection, silkworm etc.*

INTRODUCTION

Nosema bombycis naegeli is a microsporidian belonging to a ubiquitous group of eukaryotic obligate intracellular parasites, which was first identified in France by Louis Pasteur more than 100 years ago from infections with Bombyx mori L. The diagnosis of microsporidiosis has traditionally relied on microscopy for spores and transmission electron microscopy (TEM) for polar filaments and other species-specific ultra-structural features. Microscopy is widely used for pebrine monitoring in sericulture seed multiplication and production centers at 600× magnification, which is limited by specificity and sensitivity as well as difficulties in early identification. Light microscopy-based methods are faster; but still require experienced personnel for

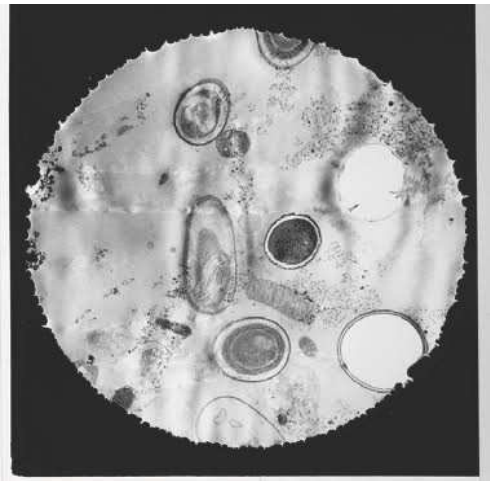

Fig.1 Nosema bombycis TEM photograph spores With one Long Polar Tube and 05 short Polar tube

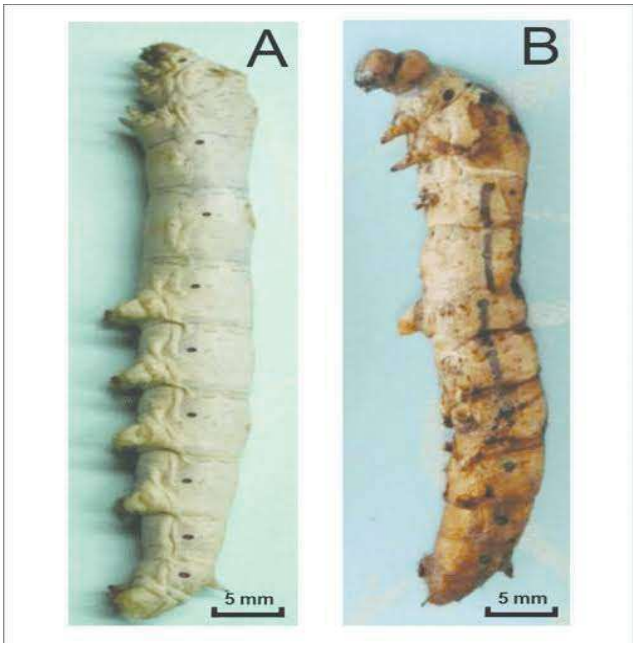

Fig.2 Pebrine disease of silkworms caused by N. bombycis

successful identification (spotting) and interpretation. Phase contrast microscopy may also be useful in laboratory examinations of microsporidia. Spore counting can be done using Neubauer's double hemocytometer and the usual time of sample handling ranges from 10 to 20 minutes.

N. bombycis produces an environmentally resistant spore, which extrudes a coiled, inner polar filament to inoculate the sporoplasm into the host cell. Microsporidian spores can be visualized using histological stains such as modified trichrome stain, alone or in combination with Gram or Warthin-Starry silver stain (Garcia, 2002). However, the inner polar filament cannot be easily identified by light microscopy in body fluids and tissues and diagnosis mostly depends on the detection of the birefringent thick spore wall. Chemo fluorescent brighteners (Calcofluor White, Uvitex 2B, Fungifluor, Fluorescent Brightener 28 and Propidium Iodide) targeting the chitin/nucleus of the spore have been used to detect microsporidians (Dai et al., 2017). Fluorescent microscopy reveals blue-stained N. bombycis spores (FB28) and red nuclei (PI); but immature spores can only be stained by PI and infection can be detected only 4–6 days after infection. Routine diagnosis based on morphology or histopathology or ultrastructural features is not always possible. Therefore, a faster, more specific, practically feasible method is needed to diagnose pebrine in silkworm seed multiplication and egg production centers.

Although microsporidians are difficult to detect, significant progress has been made in the last two decades to develop molecular (immunological/DNA based) diagnostic methods. These procedures are designed to detect either nucleic acid sequences or antigens specific for N. bombycis.

Section snippet

Purification of Nosema bombycis spores

N. bombycis spores are purified by following the method described below for developing antibodies or molecular diagnostic probes (Kawarabata and Ishihara, 1984).

- Inoculate III instar silkworm larvae with N. bombycis spores (1 × 10 7 spores/ml) orally through mulberry leaf (1 ml/100 larvae for 6 h, fresh from II molt on feed resumption)
- Rear silkworms till spinning following standard rearing conditions in controlled place
- Collect silkworm larvae (V instar, last day) or moths infected with N. bombycis

Immunodiagnostic methods

Immunoassays are used to detect and diagnose silkworm diseases, especially microsporidian infections caused by N. bombycis. Antigens are prepared from purified N. bombycis spores to develop polyclonal and monoclonal antibodies for various immunoassays.

Nucleic acid-based diagnostic methods

Nucleic acid-based detection methods use synthetic DNA primers specific and complementary to the pathogen DNA sequence. The early techniques used were based on labeled probes hybridized to N. bombycis DNA that emitted a detectable (fluorescent/radioactive) signal. These have been replaced by polymerase chain reaction (PCR), where the target DNA is bound to a specifically designed set of primers and copied repeatedly in the presence of free dNTPs by a thermo-stable polymerase enzyme.

Conclusion

The practical utility of nucleic acid-based diagnostic methods is far superior to conventional techniques in detecting N. bombycis infection with respect to sensitivity, specificity, speed and reproducibility. While the cost of these methods requires training of personnel, which may initially seem prohibitive, the underlying cost of delayed and non-specific or inaccurate diagnosis outweighs the benefits to the silk industry. Molecular detection techniques (immunology and DNA based) described.

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