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[Short Communication]

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Preliminary Study on a Microsporidian Isolate Occurring in the Lamerin Breed of the Silkworm *Bombyx mori* L. in India

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The silkworm, *Bombyx mori* L. is prone to infection of various pathogenic organisms. Pebrine, one of the deadliest disease of silkworm caused by highly virulent parasitic microsporidian, *Nosema bombycis* has been understood since long. Infections of the disease range from chronic to highly virulent and can result in complete lose to the sericulture industry. Several strains and species of microsporidians have since been isolated from the infected silkworms. A new microsporidian spore was isolated from Lamerin breed of the silkworm *B. mori* have been studied under scanning electron microscope, found to be different in spore size (length 4.36 ± 0.06 µm, width 2.14 ± 0.01 µm) and shape (ova cylindrical with slight depression) from standard strain *N. bombycis* (length 3.08 ± 0.21 µm, width 2.01 ± 0.05 µm and ovidal respectively). In immunological test, the silkworm breed Lamerin isolated micrisporidian spore does not react to different antibody (*N. bombycis*, M_{11} and M_{12}) sensitized latex particle and thus appeared to be a different strain of microsporidian parasitic to the Lamerin breed of the silkworm *B. mori*.

Key words: Microsporidian isolate, Antibody, Scanning electron microscope, Lamerin breed, Silkworm

Introduction

Microsporidia are tiny obligatory eukaryotic intracellular organisms (1 – 10 µm) are well adapted in pathogenicity, transmission, ecology, and resistance to the defense mechanism of their hosts. Insects in nearly all the taxonomic orders are susceptible to the pathogen, but over half of the susceptible insect hosts occur in two orders, Lepidoptera and Diptera. Most of the entomopathogenic microsporidia occur in genus *Nosema*, more than 150 described species found in 12 orders of insects (Becnel and Andreadis, 1999). The microsporidian *N. bombycis*, which is the type species of this genus (Sprague *et al.*, 1981) is known to infect silkworm *B. mori*, causing deadliest Pebrine disease has caused heavy losses in sericulture in Europe, as well as in Asia and America, especially in the middle of 19th century (Steinhaus, 1949).

Review of literature shows that the different microsporidian isolates have been isolated from silkworm *B. mori* (Fujiwara, 1980, 1984, 1985; Ananthalakshmi *et al.*, 1994). However, microsporidians associated with the Lamerin breed of the silkworm *B. mori*, isolated and compared with type species *N. bombycis* found different in morphology which is one of the important characteristic feature used for taxonomy (Weiser, 1961; Sprague, 1977). The present paper deals with the scanning electron microscopy, spore size and its detection by using antibody sensitized latex particles of different microsporidians available with type species *N. bombycis* as control as suggested by Mike *et al.* (1988).

Materials and Methods

Collection and characterization of microsporidian isolates
Microsporidian spores were isolated from diseased larvae of Lamerin breed of the silkworm *B. mori*, (were tentatively abbreviated as Lb_{ms}) homogenized in 0.6% K_2CO_3, filtered and the filtrate was centrifuged at 3000 rpm for 15 min to sediment the spores. The sediment was suspended in 1 ml distilled water and mixed with percoll in the ratio

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of 1:3 and centrifuged at 5000 rpm for 15 min. The spores were collected from the sediment and washed in distilled water thrice and stored as stock at 4°C for further study. The purified spores of standard strain, *N. bombycis* were collected from Silkworm Pathology Laboratory of Central Sericulture Research and Training Institute Mysore, India. The length and width of hundred mature spores were measured by ocular micrometer (Fujiwara, 1980) and mean was calculated by standard deviation. A preliminary identification was done with latex agglutination test using microsporidian spore-specific monoclonal antibodies sensitized latex kit of Yakult (Japan). The purified spores were used as antigens for the antibodies sensitized latex agglutination test. A drop of microsporidian spore suspension was mixed with equal amount of *N. bombycis*, M_{11}, and M_{12} spore specific antibodies on clean glass slide and mixed with a glass rod. Then the suspension was incubated for 10 min at room temperature and observed under Phase Contrast microscope (Nikon, AFX-DX) at 600X magnification for agglutination.

Scanning electron microscopy

Samples of purified microsporidian spores were dried at room temperature for study in the scanning electron microscope at Central Sericultural Research and Training Institute Mysore, India. The microsporidian spore samples were transferred onto double-stick cellophane tape pasted on copper stubs used for mounting specimen for scanning electron microscopy. The mounted stubs were coated with about 20 nm gold in a sputter coater (EMS-550), and viewed under a JEOL 100 CX-II electron microscope fitted with a scanning attachment (ASID-4D) scanning electron microscope (Tokyo, Japan). Micrographs were taken for determining the type. The morphology is reported to be one of the important criteria for characterization of microsprodians, as the length and width varies among different microsporidians. The observation has been compared with standard strain *N. bombycis*.

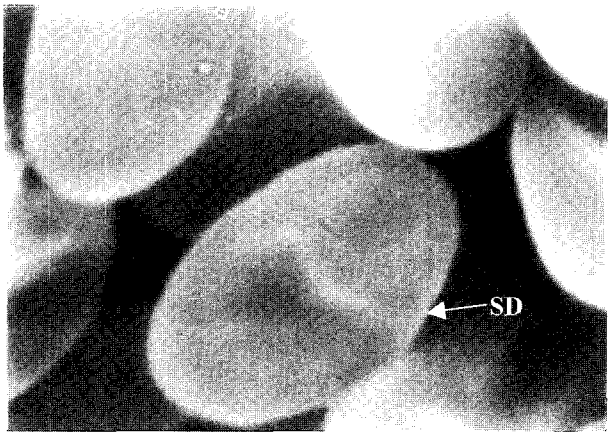
Results

Morphological characterization of microsporidian spores occurring in Lamerin breed of the silkworm *B. mori*

The length of spore was 4.36 ± 0.06 µm and width of 2.14 ± 0.01 µm which was 3.08 ± 0.21 µm and 2.01 ± 0.05 µm in *N. bombycis* respectively (Table 1). The electron micrograph indicated variation in the morphology between Lb_{ms} and *N. bombycis*. Due to electron micrographs we became aware of difference which was difficult under light microscope. The Lb_{ms} possesses ova cylindrical spore shape with slight depression at 1/4th of length of spore (Fig. 1) which was oval in case of standard strain (Fig. 2).

Table 1. Measurement of the microsporidian spore size occurring in Lamerin breed (Lb_{ms}) of the silkworm *Bombyx mori* L. with comparison to standard strain (*N. bombycis*)

Microsporidian Isolates	Form	Spore size (µm)	
		Length ± SD	Width ± SD
Lb_{ms}	Ova cylindrical	4.36 ± 0.06	2.14 ± 0.01
N. bombycis	Oval	3.08 ± 0.21	2.01± 0.05



SD: Slight Depression.

Fig. 1. SEM photograph of microsporidian isolates occurring in Lamerin breed of silkworm *Bombyx mori* L.

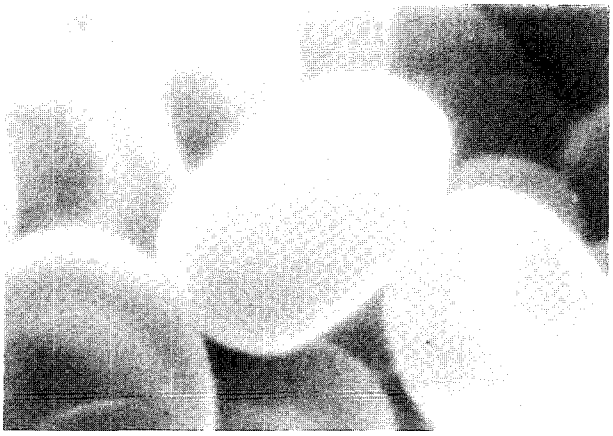


Fig. 2. SEM photograph of standard strain *Nosema bombycis*.

Antibody sensitized Latex agglutination test

In the serological agglutination test of Lb_{ms} showed a negative reaction and did not react with any of the antibodies *i.e.*, *N. bombycis*, M_{11}, M_{12}. Sensitized latex particles and appeared to be different microsporidian strain (Table 2).

Table 2. Serological reaction of the microsporidian spore occurring in Lamerin breed of the silkworm *Bombyx mori* L. to monoclonal antibody sensitized latex

Microsporidian Isolates	Monoclonal antibody to microsporidian spore		
	N. bombycis	M_{11}	M_{12}
Lb_{ms}	–	–	–
N. bombycis	+	–	–

+ : Positive reaction
– : Negative reaction

Discussion

The observation revealed that the microsporidians (Lb_{ms} and *N. bombycis*) differed in their spore size and shape. The measurement of isolated microsporidian was found different from that of the standard strain (*N. bombycis*). Electron micrograph of Lb_{ms} showed difference in spore morphology was ova cylindrical having slight depression at $1/4^{th}$ of the spore length which was oval in standard strain. On the other hand, in immmonological test when the antibody sensitized latex kit of Yakult was used the microsporidian (Lb_{ms}) does not react any of the different antibodies sensitized latex particles of the microsporidians of *N. bombycis*, M_{11}, M_{12}. Therefore it indicates that the microsporidian isolated from the Lamerin breed of the silkworm *B. mori* in India may be different strain of the microsporidian.

It is concluded that the microsporidian isolated from the Lamerin breed of the silkworm *B. mori* for the first time in India was different strain of microsporidian parasitic to the Lamerin breed of the silkworm on the basis of: (a) variation in morphology when compared to the standard strain (*N. bombycis*), (b) showed negative reaction to the antibody sensitized latex particles, and (c) larger and depression in spore size (electron micrographs). Further characterization like site of infection, pathogenicity *etc.* will be reported elsewhere.

References

Ananthalakshmi, K. V. V., T. Fujiwara and R. K. Datta (1994) First report on the isolation of three microsporidians (*Nosema* spp.) from the silkworm, *Bombyx mori* L. In India. *Indian J. Seric.* **33**, 146-148.

Becnel, J. J. and T. G. Andreadis (1999) Microsporidia in insects; in *The microsporidia and microsprodiosis.* Wittner, M. and L. M. Weiss. (eds.), pp. 447-501. ASM press, Washington, D. C.

Fujiwara, T. (1980) Three microsporidians *(Nosema spp.)* from the silkworm *Bombyx mori. J. Seric. Sci. Jpn* **49**, 229-236.

Fujiwara, T. (1984) A pleistophora like microsporidian isolated from the silkworm *Bombyx mori. J. Seric. Sci. Jpn* **53**, 398-402.

Fujiwara, T. (1985) Microsporidian from silkmoths in egg production sericulture. *J. Seric. Sci. Jpn* **54**, 108-111.

Mike, A., M. Ohwaki and T. Fukada (1988) Preparation of monoclonal antibodies to the spores of *Nosema bombycis*, M_{11} and M_{12}. *J. Seric. Sci. Jpn* **57**, 189-195.

Sprague, V. (1977) Classification and phylogeny of microsporidia; in *Comparative Path biology. Systematic of Microsporidia.* Bulla, L. A. Jr. and T. C. Cheng. (eds.), Vol. 2, pp. 1-30. Plenum Press, New York.

Sprague, V. (1981) Microspora; in *Synopsis and Classification of Living organisms.* Parker, S. P. (ed.), Vol. 1. pp. 589-594, McGraw-Hill, New York.

Steinhaus, E. A. (1949) Protozoan infections; in *Principles of insect pathology.* Brink, B. A. (ed.), pp. 592-602, McGraw-Hill, New York.

Wieser, J. (1961) Die Mikrosporidien als Parasiten der Insekten. *Monogr. Agew. Ent.* **17**, 1-149.