CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2012 Vol. (2-3) Jul.-Sept. & Oct.-Dec., pp.67-70/Melchias et al.
Research Article

CHARACTERISTIC FEATURES OF MICROSPORIDIANS ISOLATED FROM SILKWORM, *BOMBYX MORI* L

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ABSTRACT
Microsporidian spores isolated from mulberry silkworm tentatively designated as (M1and M2) are investigated and presented in this paper. The spores of M1and M2 were ovo-cylindrical in shape with 1.73 and 2.05 m in length and 1.01 and 1.47m in width respectively. However the spores of standard strain (Nosema bombycis) were oval in shape with 1.87m and 1.39m in length and width respectively. The spore length-width ratios were 1:1.71 and 1:1.39 in M1and M2 microsporidian spores, however in case of N. bombycis the spore length-width ratio is 1:1.34. The numbers of coils of polar filament were 10 in M1 however 12 coils of pf were recorded in M2 and standard strain N. bombycis. It is concluded from these observations that the M1 and M2 microsporidian spores are different from each other and also from N. bombycis.

Key Words: *Bombyx Mori, Microsporidians, Nosema Bombycis, Polar Filament, Ultrastructure*

INTRODUCTION
Insects in nearly all the taxonomic orders are susceptible to microsporidia, 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). Review of literature shows that the different microsporidian isolates have been isolated from silkworm *B. mori* in India (Bhat and Nataraju, 2004; Bhat *et. al.,* 2009a and b). The early descriptions of microsporidians were mainly based on spore morphology and lacking ultra structural details but in the recent identification, it was felt necessary to use at least a minimum of ultrastructural characters (Larson, 1988). We report herein on the characteristic features of microsopridians isolated from silkworm, *Bombyx mori* and the significant differences of the fine spore structures that serve as criterion to differentiate microsporidian species.

MATERIALS AND METHODS
Microsporidian Isolates
Spores of these microsporidia were isolated from silkworm *B. mori* by macerated infected / dead larvae and the resultant spore suspension was filtered through the wet cotton. Spores in the filtrate were purified by following the method of Sato and Watanabe, (1980).
Electron microscopy
For Scanning electron microscopical studies dried microsporidia of all microsporidians (MI, M2 and *N. bombycis*) were air dried and mounted onto the copper stubs using double sided sticky tape and coated with gold (20-mm thickness) under a sputter coater (EMS-550). The coated samples were scanned at 20kV under ASID-4D scanner attached to JEOL 100CX-11 electron microscope. For TEM studies, the spores of all the three microsporodians were fixed in 3 % (v/v) glutaraldehyde (C5H8O2) prepared in phosphate buffer saline (PBS, pH. 7.4), kept at 4 ^{0}C for 24 h, washed several times with buffer (pH. 7.2) till the odor of the fixative was completely removed. The samples were post fixed in 1% (w/v) Osmium tetra oxide (OsO_4) for 2 h, washed, dehydrated in an ascending series of alcohol of 70, 80, and 90 % (1h in each change), enbloc stained with 2 % urbanite acetate and dehydrated in absolute alcohol (100 %) for

1 h. The samples were again passed through propyle oxide (2-changes of 15 min each for clearing) and were infiltrated with araldite and propel oxide in the ratio of 1:1 for 12 h. The samples were centrifuged and the sediment was infiltrated again with fresh araldite (3-changes of 4 h each), embedded in araldite and kept at 60 ^{0}C for 48 h. Ultra thin sections 700-800 ^{0}A were double stained with Uranyl acetate and lead citrate, observed under 60 kVA (JEOL 100CX) electron microscope.

RESULTS AND DISSCUSSION

The spores were ovo-cylindrical in shape with 1.73 and 2.05 m in length and 1.01 and 1.47m in width in M1and M2 respectively (Plate 1a & b and Table 1). However the spores of standard strain (*N. bombycis*) were oval in shape with 1.87m and 1.39m in length and width respectively (Plate 1c and Table 1).

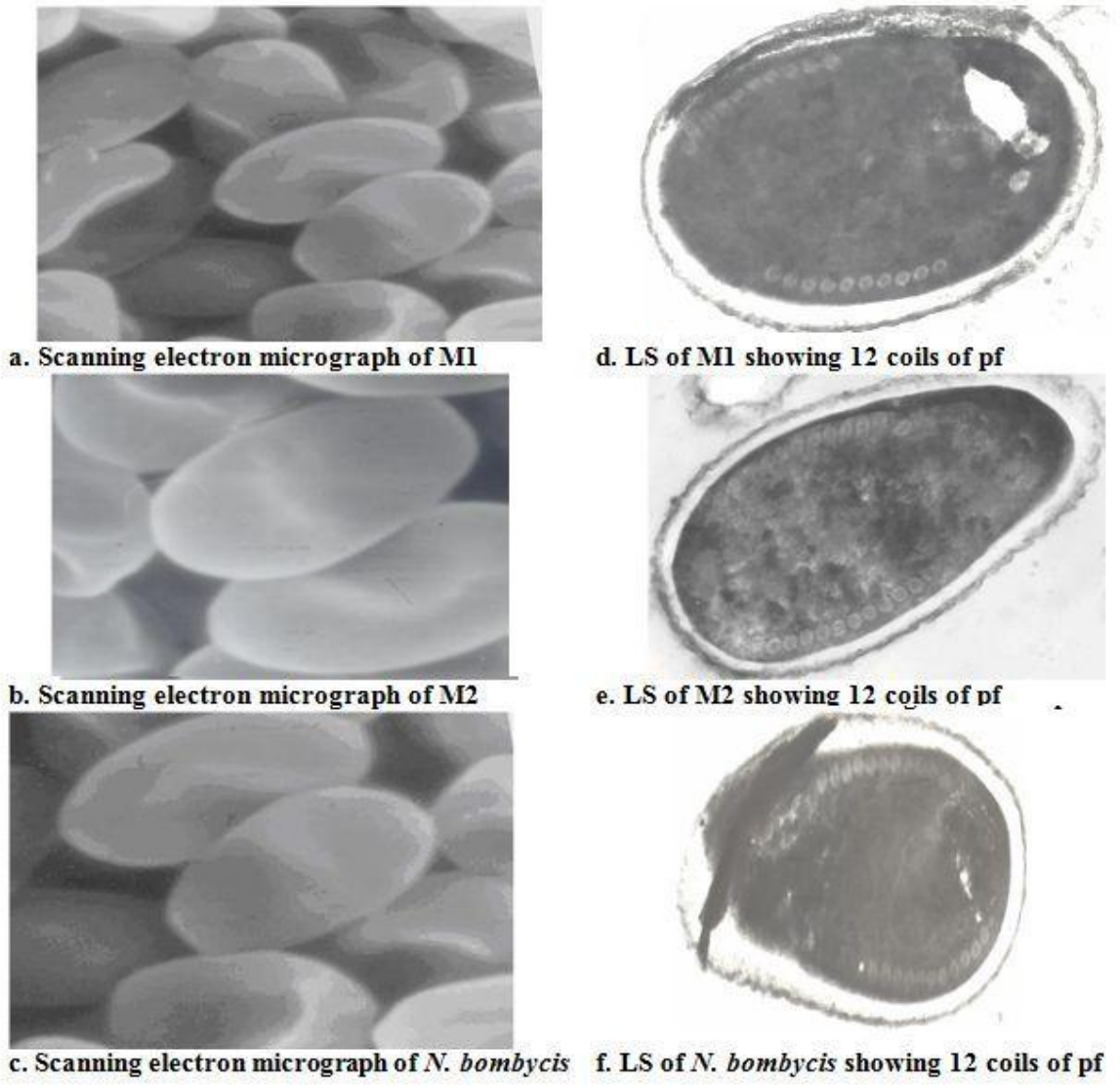

Plate 1: Electron micrographs (SEM & TEM) of three microsporidian spores (M^I, M^2 and Standard strain *N. bombycis*).

a. Scanning electron micrograph of M1 d. LS of M1 showing 12 coils of pf b. Scanning electron micrograph of M2 e. LS of M2 showing 12 coils of pf c. Scanning electron micrograph of *N. bombycis* f. LS of *N. bombycis* showing 12 coils of pf

The spore length-width ratio was 1:1.71 and 1:1.39 in M1and M2 microsporidian spores, however in case of *N. bombycis* the spore length-width ratio was 1:1.34. These microsporidian spores were found singly in their sporophorous vesicles with a thin exospore. Internal ultrastructure showed two nuclei in all the three microspridian isolates with 10 coils of polar filament (Plate 1d) however in case of M2 and *N. bombycis* 12 coils of the polar filaments have been recorded (Plate 1e&f).

Table 1: Measurement of the microsporidians and compared with that of the standard strain *N. bombycis*

Microsporidia Isolates	Spore form	Spore size (µm)		Spore length-width ratio	No. of coils of polar filament	Single polar filament coil size (µm)		Polar filament coil length-width ratio
		Length	Width			Length	Width	
M_I	Ovo cylindrical	1.73	1.01	1:1.71	10	0.41	0.36	1:1.11
M_2	Ovo cylindrical	2.05	1.47	1:1.39	12	0.97	0.91	1:1.06
N. bombycis	Oval	1.87	1.39	1:1.34	12	0.65	0.45	1:1.44

The single coil length and width were 0.41 and 0.36m and 0.97 and 0.91 in case of M1 and M2 however the *N. bombycis* it 77 was recorded 0.65 and 0.45m respectively. The polar filament length-width ratios were 1:1.11 and 1:1.06 in M1and M2 microsporidian spores. However in case of *N. bombycis* the polar filament length-width ratio was 1:1.44. Microsporidia are the most distinct, unique and complicated structure of taxonomic importance and their characterization is mainly based on the ultra structural studies. Different microsporidia from silkworm, insect pests of mulberry and pests of agricultural crops have been described based on light microscopy, which resulted in an inadequate number of species. Therefore scanning and transmission electron microscopy is essential for the characterization of microsporidia (Bhat and Nataraju, 2007). The definitive diagnosis of microsporidia required ultra structural features, because of small size and poor staining characteristics (Van den Bergh *et al.,* 1993). Early descriptions were mainly based on spore morphology and lacking ultra structural details, sometimes resulted in the unnecessary creation of new species (Malone and Mclvor, 1995). In the most recent identification key to microsporidian genera, it was necessary to use at least a minimum of ultra structural characters (Larson, 1988). The present study showed that these two microsporidians are different from each other and also from *N. bombycis*. The spores of MI and M2 microsporidia were ovo cylindrical which was oval in case of *N. bombycis*. Transmission electron microscopy is an essential for viewing internal structure and is most certain confirmation of a microsporida. The number of the polar filament coils provides useful criteria for differentiating micrisporidia and is reported to be one of the important criteria for the characterization of the microsporidians (Bhat and Nataraju, 2007). The number of coil of pf varies from 3-5 in *Encephalitozoon cuniculi* (Petri and Shiodth, 1966) to 44 in *Nosema APIs* (Scholtyseck and Danneel, 1962). The numbers of coils were 7-9 in *N. galerucellae*, 8-10 in *N. couilloudi*, 15-18 in *N. nisotrae*, 12 –14 in *N. birgi* (Toguebaye and Marchand, 1989). In conclusion, the microsporidia sp. MI and M2 described here differs from each other and also from *N. bombycis* in spore structure (shape and size) and number of coils of the polar filament. However it was difficult to place them in genus without knowing how the sporogonila divisions proceed. Moreover the relationship with genus *Nosema* is close. As there were insufficient data available on the complete life cycle of the scrounger, it could not be acknowledged at species level so we prefer at present to use the name of

collective group microsporidia as a temporary place for these microsporidians. Indeed its taxonomic position needs to be clarified in further studies.

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