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Infection and Development of Nosema sp. NIS H5 (Microsporida: Protozoa) in Several Lepidopteran Insects

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Abstract

Infection and development of Nosema sp. NIS H5, a microsporidian parasite isolated from Antheraea pernyi, in the cell line of Antheraea eucalypti were investigated, compared with those of Nosema bombycis isolated from the silkworm, Bombyx mori. No differences in the infection rate of A. eucalypti cells were observed by 48 h postinoculation between the two species of Nosema. However, the spread of infection in the cell culture with Nosema sp. became slower than that with Nosema bombycis from 96 h postinoculation onward, resulting in 16% of cell infection with Nosema sp. and 22% of cell infection with N. bombycis at 120 h postinoculation. Morphological changes in the sequence of development were almost the same in both Nosema species, but from 96 h postinoculation onward, the sporogenesis of Nosema sp. was slower than that of N. bombycis. Infectivity test to 13 lepidopteran species revealed that N. bombycis was infectious to Bombyx mori, Bombyx mandarina, Antheraea pernyi, Antheraea yamamai, Hemerophila atrilineata, Spilarctia imparilis, Hyphantria cunea, Glyphodes pyroalis, Leucania separata, Spodoptera litura and Pieris rapae crucivora, but not infectious to Lymantria dispar and Euproctis similis. On the contrary, Nosema sp. was infectious to these 12 lepidopteran species except for B. mori, and highly infectious to L. separata, inducing a lethal infection. Thus, it is concluded that Nosema sp. is not infectious to B. mori and has a wide host range of lepidopteran species, suggesting that Nosema sp. could become a very promising agent for the microbial control of insect pests, especially in sericultural areas.

Discipline: Sericulture/Pathology
Additional key words: silkworm, Nosema Bombycis, host range, lepidopteran species

Introduction

The microsporidia pathogenic to lepidopteran insects display a wide host range and the dissemination of the disease easily occurs by oral and transovum infection, suggesting that microsporidia could become useful agents for the microbial control of insect pests. However, most of the microsporidia show a low virulence to insect pests but a high pathogenicity to the silkworm, and general applications of microsporidia for microbial control are still limited[7].

In the present study, *Nosema* sp. NIS H5[6], originally isolated from diseased *Antheraea pernyi*, was investigated for infection and development in cultured *Antheraea eucalypti* cells, compared with *Nosema bombycis* isolated from the silkworm, *Bombyx mori*. Furthermore, the infectivity and pathogenicity of both *Nosema* species to 13 lepidopteran insects were determined.

Materials and methods

1) Microsporidian spores

Two microsporidia were used: one was *Nosema* sp. NIS H5 isolated from *Antheraea pernyi* (size of spore: ca. 3.9 × 1.7 μm) and the other was *Nosema bombycis* NIS H3 (size of spore: ca. 3.7 × 2.1 μm) (Fig. 1). Both microsporidia were cloned from their original strains stocked at the National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki[2].

2) Cell line

An *Antheraea eucalypti* cell line (Grace[1]) was

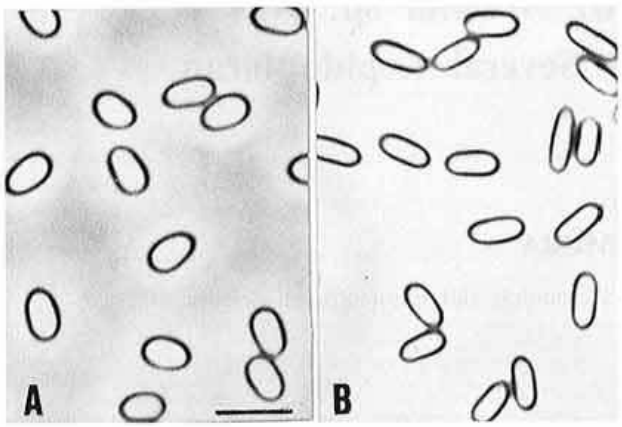

Fig. 1. Mature microsporidian spores
A: *Nosema bombycis* NIS H3.
B: *Nosema* sp. NIS H5.
Bar = 5 μm.

provided by Dr. T. Kawarabata, Kyushu University, Fukuoka. It was maintained at 26°C in Grace's medium supplemented with 5% fetal bovine serum (GIBCO) and 5% of heat inactivated (60°C for 15 min) silkworm hemolymph.

3) Inoculation of microsporidia

Microsporidian spores were purified by repeated Percoll (Pharmacia) density gradient centrifugation (73,000 g, for 30 min) and stored at 5°C until use. To infect the cell culture, an equal volume of 0.2N KOH solution was added to the spore suspension, which was then incubated at 25°C for 30 min. Subsequently, the spore suspension was mixed thoroughly with an insect cell suspension at a rate of 10 spores per cell. Each inoculated cell suspension was incubated at 26°C for 1 h. After the cells had settled to the bottom of the flask, the supernatant was replaced with fresh medium.

The infectivity of *N. bombycis* and *Nosema* sp. was tested for 13 lepidopteran insects, such as *Bombyx mori, Bombyx mandarina, Antheraea pernyi, Antheraea yamamai, Hemerophila atrilineata, Lymantria dispar, Euproctis similis, Spilarctia imparilis, Hyphantria cunea, Glyphodes pyroalis, Leucania separata, Spodoptera litura,* and *Pieris rapae crucivora.*

On the 2nd day of the 1st instar, the larvae of each insect species were fed for 6 h with leaves of the host plant which had been smeared with a spore suspension (10^8 spores/mL), and thereafter they were supplied with untreated leaves. In the case of *Leucania separata* reared on artificial diet (Nihon Nosan Kogyo), larvae immediately after hatching or larvae at 10 days after hatching were fed for 48 h with the artificial diet which had been contaminated with 0.05 mL of spore suspensions (10^3–10^7 spores/mL) per 1 g of diet.

4) Observation

For the light microscopic observation of the growth and development of microsporidia in cultured cells, the samples of infected cultures were smeared on glass slides, dried, fixed in absolute methanol, and stained with Giemsa at pH 6.8.

To determine the infection of microsporidia in insect larvae, tissues from the tested larvae at 10 days postinoculation or the dead larvae were smeared on glass slides, stained with Giemsa and examined by light microscopy for microsporidian multiplication.

Results and discussion

1) Multiplication of N. bombycis *and* Nosema *sp. in* A. eucalypti *cells*

At 1 h postinoculation of *N. bombycis*, about 5% of *A. eucalypti* cells were infected with sporoplasms which had emerged from the spores primed with KOH solution. The infection started to spread in the culture at 48 h postinoculation, and the percentage of infected cells reached 22% at 120 h postinoculation.

The percentage of infected *A. eucalypti* cells at 1 h postinoculation of *Nosema* sp. was about 5%, the same value as in the case of *N. bombycis* infection, and then a moderate increase in the number of infected cells occurred at 48–96 h postinoculation. No further increase in the number of infected cells was observed after a plateau was reached at 96 h postinoculation. The percentage of infected cells was 16% at 120 h postinoculation.

In the *A. eucalypti* cells infected with *N. bombycis*, the sporoplasm grew into a schizont at 18 h postinoculation and multiplication by binary fission of the schizont was observed at 24 h postinoculation, as shown in Table 1. At 48 h postinoculation, the production of secondary infective forms took place and sporoplasm-like microsporidian parasites were observed in some of the *A. eucalypti* cells. From 96 h postinoculation onward, sporogenesis occurred and mature spores were recognized at 192 h postinoculation.

The sequence of development of *Nosema* sp. in the *A. eucalypti* cells was almost similar to that observed in *N. bombycis* infection (Table 1). However,

the sporogenesis of *Nosema* sp. took much time and mature spores were produced from 216 h postinoculation onward.

These results indicated that *N. bombycis* is more pathogenic to cultured *A. eucalypti* cells than *Nosema* sp.

2) Infectivity of N. bombycis *and* Nosema *sp. to lepidopteran insects*

Tests of peroral inoculation of microsporidian spores to 13 lepidopteran insects revealed that *N. bombycis* spores were highly infectious to *B. mori, B. mandarina, A. pernyi, A. yamamai, H. atrilineata, S. imparilis, H. cunea, G. pyroalis, L. separata, S. litura* and *P. rapae crucivora*, but not infectious to *L. dispar* and *E. similis* (Table 2). The pathogenicity to *B. mori, B. mandarina, L. separata* and *P. rapae crucivora* was high as all the tested larvae were killed by 8 days postinoculation.

On the other hand, *Nosema* sp. spores were infectious to these 12 tested insects except for *B. mori* (Table 2). The pathogenicity to *A. yamamai* and *L. separata* was high as all the tested larvae were killed by 7 days postinoculation, but the infectivity to *E. similis* and *H. cunea* was low as the percentage of infection was less than 50%.

As mentioned above, *N. bombycis* was not infectious to *E. similis* and *L. dispar*, while *Nosema* sp. was not infectious to *B. mori*. These results were almost the same as the results of the tests performed by Hirose[3,4] and Hirose & Ueda[5]. Thus, *Nosema* sp. was different from *N. bombycis* in host range, infectivity and pathogenicity to lepidopteran insects.

3) Pathogenicity of Nosema *sp. to* L. separata

As described previously, *Nosema* sp. showed a

Table 1. Development of *Nosema bombycis* and *Nosema* sp. in cultured *Antheraea eucalypti* cells

Microsporidia	Developmental stage	Hours postinoculation
Nosema bombycis	Sporoplasm	1
	Schizont	18
	Secondary infective form	48
	Immature spore	96
	Mature spore	192
Nosema sp.	Sporoplasm	1
	Schizont	18
	Secondary infective form	48
	Immature spore	96
	Mature spore	216

Table 2. Infectivity of *Nosema bombycis* and *Nosema* sp. to lepidopteran insects

Insect species	Number of larvae infected with	
	Nosema bombycis	*Nosema* sp.
Bombyx mori	10	0
Bombyx mandarina	10	7
Antheraea pernyi	8	10
Antheraea yamamai	7	9
Hemerophila atrilineata	10	7
Lymantria dispar japonica	0	6
Euproctis similis	0	5
Spilarctia imparilis	10	8
Hyphantria cunea	9	4
Glyphodes pyroalis	10	6
Leucania separata	10	10
Spodoptera litura	10	7
Pieris rapae crucivora	10	7

Inoculation: Leaves of host plant which had been smeared with a suspension containing 10^8 spores/mL were fed for 6 h to 1st-instar larvae on the 2nd day of hatching.
Number of tested larvae: Ten larvae each.

Table 3. Pathogenicity of *Nosema* sp. to the larvae of *Leucania separata*

Time of inoculation	Inoculated suspension (spores/mL)	Number of larvae which died													
		Days postinoculation													
		2	3	4	5	6	7	8	9	10	11	12	13	14	15
Day of hatching	10^3									1	1	2	2	1	
	10^5				2	1	1	3	2	1					
	10^7	7	1	1	1										
Ten days after hatching	10^3														*
	10^5								2	3	1	3	1		
	10^7					1	1	1	1	2	3	1			

Inoculation: Artificial diet contaminated with spore suspensions at different concentrations (10^3–10^7 spores/mL) was fed for 48 h to the larvae.
Number of tested larvae: Ten larvae each.
* All of the tested larvae grew into the prepupal stage.

strong pathogenicity to *L. separata*. Artificial diets contaminated with *Nosema* sp. spore suspensions at different concentrations (10^3–10^7 spores/mL) were fed to *L. separata* larvae immediately after hatching, resulting in the infection and death of all the tested larvae (Table 3). The time of lethal infection depended on the spore concentration of the diet which had been fed to the larvae. The larvae inoculated with spore suspensions at higher concentrations died faster. For example, the larvae inoculated with a suspension containing 10^7 spores/mL died at 2–5 days postinoculation, whereas the larvae which received a suspension containing 10^3 spores/mL died at 10–14 days postinoculation.

An inoculation test was also performed to the larvae at 10 days after hatching. All the tested larvae fed on the diet treated with a suspension containing 10^7 spores/mL died at 6–12 days postinoculation, whereas almost all of the larvae inoculated with a suspension containing 10^3 spores/mL survived and grew into the prepupal stage (Table 3). Thus, the occurrence of lethal infection depended on the concentration of the spore suspension inoculated as well as the larval age when spores were inoculated. The larval susceptibility to the spore inoculation decreased with the larval age.

The present study revealed that *Nosema* sp. had a wide host range of lepidopteran species and was highly infectious to *L. separata* as it induced a lethal infection in the younger larvae, but was not infectious to *Bombyx mori*. Accordingly, *Nosema* sp. could become a useful agent for the microbial control of insect pests, especially in sericultural areas. However, further studies on transovum transmission of *Nosema* sp. to the subsequent generation of insect pest population are required to develop a method of microbial control using the microsporidian parasite.

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