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Transmission and Infectivity of Spores of *Burenella dimorpha* (Microsporida: Burenellidae)

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Burenella dimorpha infects the tropical fire ant, *Solenopsis geminata*, producing two morphologically distinct types of spores. A binucleate, nonpansporoblast membrane-bounded (NPMB) spore develops in and destroys the hypodermis, rupturing the cuticle in the pupal stage. A uninucleate, pansporoblast membrane-bounded (PMB) spore develops in the fat body. Adult ants cannibalize ruptured pupae but do not ingest spores. Instead, the spores and particulate foods are diverted to the infrabuccal cavity, formed into an infrabuccal pellet, and fed to fourth-instar larvae only. This larval instar is the only stage in the life cycle of *S. geminata* that is vulnerable to infection. NPMB spores are infective, but PMB spores do not extrude their polar filaments in the larval gut and are expelled in the meconium upon pupation.

KEY WORDS: *Burenella dimorpha*; *Solenopsis*; fire ants; microsporida.

INTRODUCTION

Burenella dimorpha, originally described by Jouvenaz and Hazard (1978), is a microsporidium which parasitizes the tropical fire ant, *Solenopsis geminata*. It produces two morphologically distinct types of spores. Binucleate, nonpansporoblast membrane-bounded (NPMB) spores develop from disporous sporonts in the hypodermis; uninucleate, pansporoblast membrane-bounded (PMB) spores develop in octets from multinucleate sporonts in the fat body. Infection and destruction of the hypodermis produces characteristic clear areas in the occiput, petiole, and gaster of the pupa. As the infection progresses, the clear areas increase in size, and the cuticle becomes very fragile, and eventually ruptures. The pupa is then cannibalized by workers; we have observed this in laboratory colonies.

Suspensions of spores of *B. dimorpha*

containing both types of spores are infective per os for *S. geminata* and certain other *Solenopsis* spp. (Jouvenaz and Hazard, 1978). However, attempts to separate the spore types by density gradient centrifugation were unsuccessful (the pansporoblast membrane of *B. dimorpha* is very delicate, rupturing on dissection of the host, and the free PMB spores are nearly identical in bouyant density to NPMB spores). Therefore, Jouvenaz and Hazard were unable to determine which or if both spore types are infective. They described this microsporidium as a dimorphic species on the basis of light microscopy studies of the life cycle, but could not confirm by experimental feeding tests that both spore types were produced by the same organism.

Petralia and Vinson (1978) reported that adult workers of the red imported fire ant, *Solenopsis invicta*, feed liquid food to larvae in all instars but feed solid foods to fourth-instar larvae only. The worker places a pellet of solid food from her in-

frabuccal cavity on the praesaepium ("breadbasket") of a larva, and the larva consumes the pellet. We observed (unpubl.) that adult workers are able to screen very small ($<10\ \mu\text{m}$) particles from aqueous or oil suspensions. These findings suggested a cycle of infection from ruptured pupae to fourth-instar larvae vectored mechanically by adults via the infrabuccal pellet. This study was conducted to (1) determine the mode of transmission of *B. dimorpha*, (2) to determine whether one or both spore types are infective, and (3) to experimentally verify that this microsporidium is indeed a dimorphic species.

MATERIALS AND METHODS

A suspension of spores was mixed with boiled egg yolk and offered to a small, healthy colony of *S. geminata*, which was allowed to feed ad lib for 24 hr. Workers were trapped while feeding, held 1 hr, killed by freezing, sectioned into the three body parts, and examined by phase microscopy to determine whether spores were ingested or diverted to the infrabuccal cavity (microsporidian spores are visible in slightly compressed, intact, isolated fire ant body segments at $300\times$).

Eight fresh infrabuccal pellets that had been placed by workers on the praesaepia of fourth-instar larvae were removed and examined by phase microscopy for spores.

After 24 hr, 109 fourth-instar larvae were removed from the nest and held in a small isolation nest with conspecific workers that had never been exposed to spores. These workers functioned as nurses. After 21 days, the immatures, which had pupated, were examined for infection. A group of 79 pre-fourth-, primarily third-, instar larvae were also removed and held in a separate nest with conspecific workers until they eclosed as adults.

A suspension of mature spores of both types was prepared by homogenizing diseased pupae in distilled water with a glass tissue grinder and centrifuging the extract in a discontinuous gradient (100, 75, 50, and

25%) of Percoll¹ (Pharmacia Fine Chemicals) for 20 min at ca. 10,000 g. This procedure produced a clean suspension of spores of both types, almost all of which appeared to be mature. These spores were fed to fourth-instar larvae in a paste prepared from finely powdered (mortar and pestle) dry baby cereal and spore suspension. With a blunted and flattened insect pin, infrabuccal pellet-sized quantities of the paste were placed on the praesaepia of the larvae, which were then held in a small isolation nest until pupation. Adult workers were introduced to care for these larvae ca. 4 hr after feeding. When the larvae pupated, we recovered 20 meconia and examined them by phase microscopy to determine whether the spores had extruded their polar filaments.

A suspension of NPMB spores only was obtained by selecting diseased pupae which, on the basis of gross pathologic manifestations, were estimated to harbor some mature NPMB spores but no mature PMB spores (NPMB spores develop before PMB spores). Wet mounts of individual pupae were examined by phase microscopy, and those that appeared free of mature PMB spores were washed from the slides with distilled water, pooled, and cleaned and concentrated by centrifugation. An examination of 10,000 spores individually in a diluted aliquot (adjusted to 0–5 spores/field) and careful scanning of the concentrated suspension confirmed that no PMB spores were present in this preparation.

The suspension of NPMB spores was mixed with boiled egg yolk and offered to a small, healthy colony of *S. geminata*. After 20 days, 25 pupae which exhibited signs of advanced infection were individually homogenized in ca. 0.5 ml of water and examined by phase microscopy (0–6 spores/field) to determine the ratio of the spore

¹ Mention of a commercial or proprietary product does not constitute a recommendation or an endorsement of the product by the U.S. Department of Agriculture.

types in the first 200 spores seen. We also examined 25 infected pupae from a laboratory colony that had been infected with a crude suspension of mixed spores for routine propagation of the parasite.

RESULTS

S. geminata adults did not ingest spores of *B. dimorpha*; instead, they diverted them to the infrabuccal cavity. One hour after exposure to food-containing spores, 100 workers of 160 examined contained spores in their infrabuccal cavities; none contained even a single spore in the digestive tract.

All eight infrabuccal pellets removed from fourth-instar larvae shortly after deposition by workers contained numerous spores.

Only fourth-instar larvae became infected with *B. dimorpha*. Seventy-one (65.1%) of the one hundred and nine exposed to spores as fourth-instar larvae became infected; none of the 79 pupae exposed to spores as third- or earlier-instar larvae became infected.

NPMB spores were infective per os for *S. geminata* and produced infections with characteristic gross pathology and both spore types. Furthermore, the spore types were produced in normal ratios. Pupae fed the suspension of NPMB spores as larvae contained $28.6 \pm 10.2\%$ ($r = 16-48\%$) PMB spores. Pupae fed the suspension of both spore types as larvae contained $30.8 \pm 7.8\%$ ($r = 16-43\%$) PMB spores.

In the meconia of larvae fed the suspension of mature spores of both types, 1723 (97.1%) of the 1774 spores found had extruded their polar filaments. The 51 (2.9%) NPMB spores that had not extruded their polar filaments were probably not quite mature, as they were very slightly less refractile or less deep amber internally than mature spores.

PMB spores did not appear to be infective per os for *S. geminata*. Examination of meconia showed that few if any PMB spores had extruded their polar filaments while in the larval gut. A total of 274 ma-

ture, nonextruded PMB spores were seen in the 20 meconia, but only one body was seen which strongly appeared to be an extruded PMB spore. Five additional bodies were seen that resembled extruded PMB spores but almost certainly were not. The meconia contained occasional empty walls of ingested or gut unicellular fungi, some of which superficially resembled extruded PMB spores; extruded NPMB spores were distinct.

DISCUSSION

The cycle of *B. dimorpha* infection within an ant colony may be summarized as follows: NPMB spores develop in the hypodermis, which is destroyed, producing clear areas in the heads, petioles, and gasters of pupae. As the infection progresses, the cuticle becomes very fragile and eventually ruptures. The adult ants cannibalize these ruptured pupae but do not ingest the spores. Instead, the spores, together with other particulate matter, are diverted to the infrabuccal cavity and formed into an infrabuccal pellet. This pellet is expelled and placed on a specialized anteroventral area, the praesaepium of fourth-instar larvae. The praesaepium, which bears spines specialized for holding solid food while the larva feeds, is absent from earlier instars, which are fed only liquid. Because of this method of feeding, the fourth-instar larva is the only stage which is vulnerable to infection. Both spore types are ingested, but only the NPMB spore is infective. The PMB spores are expelled unextruded in the meconium upon pupation.

These results confirm that *B. dimorpha* is indeed a dimorphic microsporidium, since ingestion of NPMB spores only resulted in typical infection in which both spore types were produced, and in normal ratios.

The function of the PMB spore remains unknown. A most attractive hypothesis is that it either infects an alternate host or is primed in the gut of a mechanical vector for extrusion upon subsequent ingestion by ant larvae. Either would explain the mode of

intercolonial transmission of the infection (fire ants are territorial and aggressive toward conspecific ants). Many candidate species exist for the role of vector; a large and varied arthropod fauna is associated with fire ants. Collins and Markin (1971) listed 52 species of insects that have been collected from fire ant nests; other invertebrates also occur. At least some of these organisms have symbiotic relationships with fire ants and are known to travel between fire ant nests (Wojcik, 1975).

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