Inoculation of spruce budworm cell cultures with the microsporidium, *Nosema whitei*

by

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Abstract

Spores of the microsporidium Nosema whitei were successfully primed to germinate in vitro in tissue culture medium, and inoculated into the spruce budworm cell line IPRI-CF-124. The spores could be treated with the disinfectant Zephiran and still germinate, however to prevent premature germination spores had to be taken from living infected larvae. Many of the spores germinated within 5-10 min after they were introduced into the cell cultures. The sporoplasms were present in the medium for up to 8 days, and in a few cases some of the germinated spores appeared to be phagocytosed by the cells. However, multiplication of the microsporidium in the cells was not observed. Although this cell line did not get infected with N. whitei it is possible others might.

Résumé

Des spores de la Microsporidie Nosema whitei ont pu être apprêtées pour germer in vitro dans un milieu de culture de tissu, puis inoculées dans la lignée IPRI-CF-124 de cellules de la Tordeuse des bourgeons de l'Epinette. Les spores, même traitées au désinfectant Zephiran, pouvaient encore germer; toutefois, afin de prévenir une germination trop hâtive, il a fallu prélever les spores sur des larves vivantes infectées. Une bonne quantité de spores ont germé en l'espace de 5 à 10 minutes après leur introduction dans les cultures de cellules. Les sporoplasmes sont demeurés dans le milieu jusqu'à 8 jours et en certains cas, quelques-unes des spores germées semblaient phagocytées par les cellules. Cependant, on n'a observé aucune multiplication de la Microsporidie dans les cellules. Bien que cette lignée de cellules n'ait pas été infectée par N. whitei, il est possible que d'autres auraient pu l'être.

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Introduction

Many microsporidia are important debilitating pathogens and play a major role as natural control agents of forest pest insects. In recent years the use of tissue cultures in the investigations on microsporidia has become increasingly important (Ishihara and Sohi 1966; Ishihara 1969; Kurtti and Brooks 1977; Sohi and Wilson 1976; Wilson and Sohi 1977). One of the major problems in successfully infecting tissue cultures with microsporidia has been the priming of spores for germination *in vitro*. In the host, spore germination occurs in the gut as a result of high pH and, possibly, enzymatic action. *In vitro*, spores are generally induced to germinate by alkali treatment (Ishihara and Sohi 1966; Kurtti and Brooks 1977) but air dried spores of *Nosema whitei* Weiser, were induced to germinate by rehydration (Kramer 1960). To our knowledge *N. whitei* has not been grown in tissue culture cells. The purpose of these experiments was to determine if the spores of this microsporidian would germinate in culture medium and if the released sporoplasms would infect the spruce budworm cells.

Materials and Methods

A continuous cell line, IPRI-CF-124, started from minced neonate larvae of spruce budworm (Sohi 1973), was passaged *in vitro* 316 times before use in these experiments. The stock cultures were grown in 25 cm^2 polystyrene disposable flasks, and the experimental cultures in glass leighton tubes. Cells were grown in Grace's insect tissue culture medium (Grace 1962) supplemented with fetal bovine serum (15%), tryptose broth (0.25%) and Gentamicin (100 µg/ml). Gentamicin inhibits bacterial growth but it does not interfere with microsporidian infection (Sohi and Wilson, unpublished).

Nosema whitei spores were obtained from live infected larvae of the red flour beetle, *Tribolium castaneum* (Herbst), because spores from dead larvae germinated as soon as they came in contact with the aqueous pretreatment solutions. It should be noted that even with spores from living larvae, some germination occurred before pretreatment was complete. Infected larvae were homogenized in distilled water with a tissue homogenizer, and filtered through double layers of cheesecloth. After the spores were pelleted by centrifugation (1840 g for 10 min), the supernatant was discarded and the spores resuspended in a 0.0002% (1:1000 v/v of 21.3% Zephiran in distilled water) aqueous solution of Zephiran chloride (benzalkonium chloride) for 5 min to kill contaminants, such as bacteria and yeast. The spores were washed three times with Grace's tissue culture medium to remove the toxic effect of Zephiran on cells. After the final rinse, a 0.3-0.5 ml aliquot of a suspension of ca 10^{10} spores/ml was smeared on a cover glass (No. 0, 10 x 50 mm)

and allowed to dry under aseptic conditions in an Edgegard laminar flow hood (The Baker Comp. Inc., Sanford, Maine) for 1 1/2-2 hr. Cover glasses were then placed in leighton tubes, and 1 ml of cell suspension containing 5 x 10^5 cells was added. The cell cultures were incubated at 28°C. The cover glasses were removed from the culture tubes 1, 4 and 8 days after inoculation, air dried, fixed in absolute methanol for 2 min and stained for 45 min with Giemsa diluted 1:30 in distilled water. After staining, the cover glasses were rinsed in tap water for 3-4 sec, dried and mounted on microscope slides with Harleco's mounting media. The stained preparations were examined using bright field and oil immersion objective.

Results and Discussion

Microscopic examination by phase contrast revealed that many of the spores germinated shortly (5-10 min) after being placed in the culture media. Sporoplasms, polar filaments and germinated spores were readily seen in the stained preparations (Fig. 1 and 2). The sporoplasms were present in the culture medium for up to 8 days (termination of experiment); however, it was not possible to determine if they remained alive. No division of the sporoplasm was observed. In a few cases some of the germinated spores appeared to be phagocytosed by the cells (Fig. 3).

Spores must be sterilized before they can be put into tissue culture. These tests demonstrated that spores of N. whitei taken from dead larvae germinate as soon as they come in contact with the

sterilizing agent, such as Zephiran; the sporoplasm would be killed by the toxic effect of such chemicals. Therefore spores from living larvae had to be used. As a result of our experimentation we demonstrated that spores obtained from living larvae could be sterilized without inducing substantial germination and that these spores (after dehydration) would germinate when in contact with tissue culture medium. Although IPRI-CF-124 cell line did not become infected with N. whitei, it is possible by using this method that other cell lines might.

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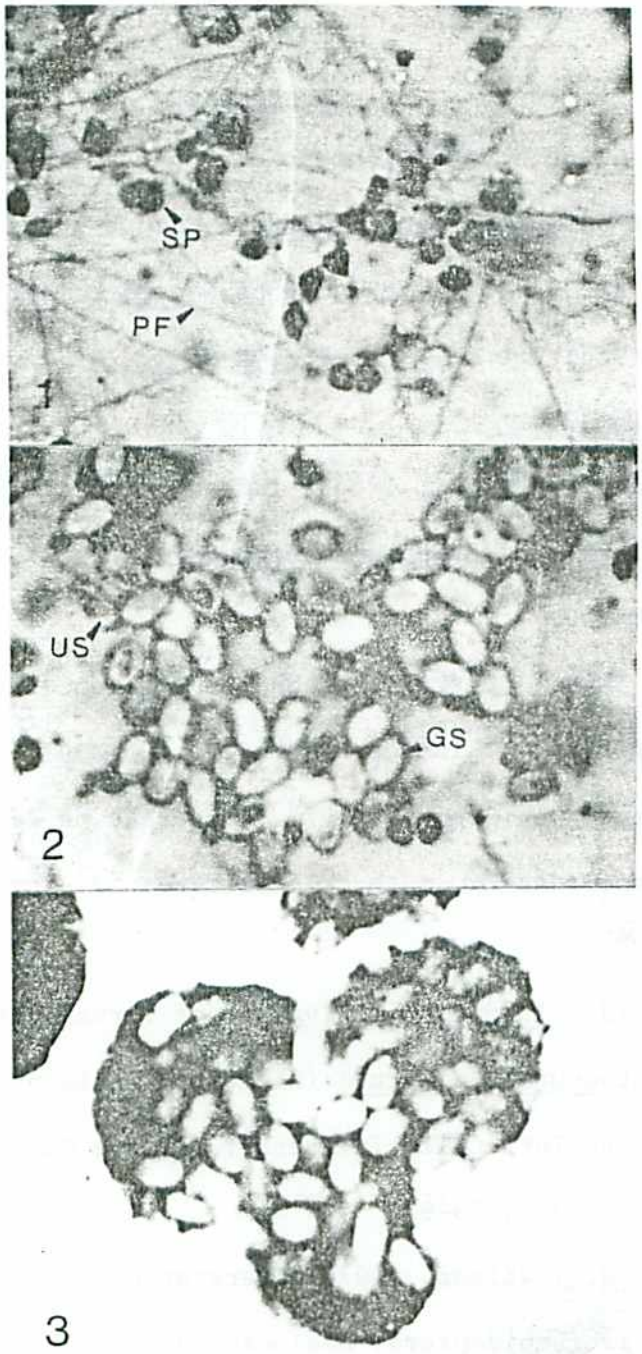

Figures 1 - 3. N. whitei in C. fumiferana tissue culture (1600X). Material was fixed in methanol and stained with Giemsa.

Fig. 1, sporoplasm (SP) and polar filaments (PF).

Fig. 2, germinated (GS) and ungerminated (US) spores.

Fig. 3, Spores phagocytosed by tissue culture cells.