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A TECHNIQUE FOR STAINING THE ENDOSPORES OF *PASTEURIA PENETRANS*

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The following simple staining technique increases the ease of detection and counting of endospores of *Pasteuria penetrans* (Sayre & Starr, 1985) adhering to the second stage larvae (L_2) of species of *Meloidogyne*.

Adhering endospores were stained to varying degrees by a range of histochemical stains. The most satisfactory of these was Brilliant Blue G (BBG), obtained from Sigma (Catalogue No. B-1131). This stain has a molecular weight of 854.04 and should not be confused with the closely related Brilliant Blue R (BBR) (Sigma B-0630)

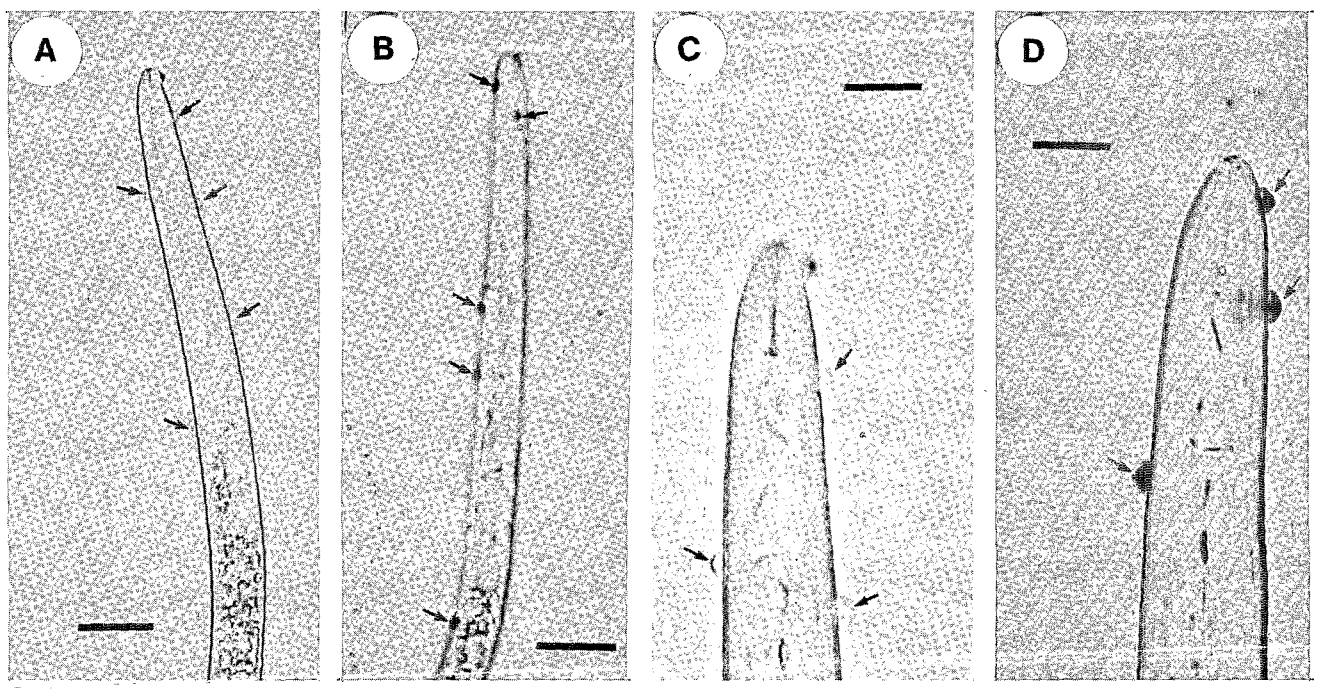

Fig. 1. A : Part of a living unstained infective second stage larva (L_2) of *Meloidogyne hapla* with attached spores of *Pasteuria penetrans* (see arrows) viewed under normal (bright field) illumination; B : Similar to A but the spores on this specimen were stained with Brilliant Blue G (BBG) and show up much more clearly (see arrows) than those in A; C : Anterior part of A at higher magnification under oil immersion showing unstained spores (arrows); D : Anterior part of B at higher magnification under oil immersion showing spores stained with BBG (arrows) (Bars represent, A, B : 25 µm; C, D : 10 µm).

which has a molecular weight of 825.986 and which is a less satisfactory stain for the endospores has been shown to stain secretions from the amphids excretory pore of the L_2 of *M. incognita* (Premachandran *et al.*, 1988. These arylmethane acid dyes are not toxic to the nematodes which will swim about in a dilute solution of BBG for several days without any apparent ill effects.

For convenience of application, the stain, which is only slightly soluble in cold water, is made up as a 1 mg/ml solution in distilled water, mixed with the aid of a magnetic stirrer for 1 hr at 23°, centrifuged at 500 G for 15 mn and stored in a 5 ml hypodermic syringe to facilitate dispensing. It is best used fresh although it is still effective after several weeks storage in the syringe at 23°.

A drop of this stain is added to a drop containing the nematodes with adhering endospores and a coverslip is applied and sealed with nail varnish. The dye commences to stain the spores within a few minutes and intensifies over several hours showing them up in marked contrast to spores on unstained material (Fig. 1). The dye also stains free unattached spores and stained spores do not lose their capacity to adhere to nematodes.

If an immediate count is required the nematodes may be killed by heating gently on a hot plate although this dye makes counting of spores on living material much easier.

Alternatively the dye, which is more soluble in alcohol than in water, may be made up in methanol : acetic acid : water (4 : 1 : 5) as recommended by Premachandran *et al.* (1988) for their histochemical studies on nematode secretions.

There is some confusion over the nomenclature of these dyes when the term Coomassie is used. In H. J. Conn's Biological Stains (Lillie, 1977) BBG is listed as Coomassie Brilliant Blue G-250 and BBR is listed as Coomassie Brilliant Blue R-250. Both these dyes belong to the aminotriarylmethane group of dyes. However, the dyes Coomassie Fast Blue BL and GL, also known as Acid Milling Blue G, are quinone-imine dyes with molecular weights of 670.703 and 700.734 respectively. They also stain the endospores of *Pasteuria penetrans* but not as uniformly as does BBG. The arylmethane acid dyes are specific for protein and have been used to stain proteins fractionated by gel electrophoresis.

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SEM DATA ON *BRACHYDORUS SWARUPI* KOSHI, RASKI & SOSAMMA, 1981, AND CONSIDERATIONS ON THE TAXONOMIC POSITION OF THE GENUS *BRACHYDORUS* DE GUIRAN & GERMANI, 1968 (NEMATA : DOLICHODORIDAE)

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Luc and Fortuner (1987) reviewed the family Dolichodoridae Chitwood, 1950 and the three genera comprising that taxon. They concluded *Dolichodorus* Cobb, 1914 and *Neodolichodorus* Andrássy, 1976 should be considered distinct and valid. The proposal of *Brachydorus* as a new genus based on its monotypic species, *B. tenuis*, described by de Guiran and Germani (1968), at first appeared justified, but the later description of *B. swarupi* Koshi, Raski & Sosamma, 1981 raised some questions. Its greater length (up to 2.3 mm), longer stylet (up to 35 µm), and shorter female tail (c' = 3.8-5.0) were intermediate between the type species, *B. tenuis*, and the generic limits of *Dolichodorus* and *Neodolichodorus*. Besides, some species of both *Dolichodorus* and *Neodoli-*

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