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DIFFERENTIAL STAINING OF "EXCRETORY" SYSTEM MUCINS OF *HETERODERA SCHACHTII* J_2-JUVENILES

by
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Summary. "Excretory" system mucins of *Heterodera schachtii* J_2-juveniles were induced to leave the secretory vesicles and gland cells. Mucin components were then differentially stained with aniline blue. The mucins were composed of a darker staining central core and a lighter peripheral region. The peripheral region decomposed during the experiments whereas the core appeared intact. It is hypothesized that the core has a higher protein content than the peripheral region.

Nematodes possess several gland cells which are located in chemosensilla and in the so-called "excretory" system. Secretions of chemosensilla and of the "excretory" system can be induced to leave the secretory vesicles and gland cells by a method described by Premachandran *et al.* (1988). The amphidial and "excretory" system secretions are composed of 0-linked glycoproteins (or mucins) in *Heterodera schachtii* males (Aumann, 1994). The aim of the present study was to differentially stain components of the "excretory" system secretions of *H. schachtii* Schm. J_2-juveniles (J_2).

Materials and methods

H. schachtii J_2 were obtained from monoxenically grown cysts (Wyss and Zunke, 1986) by incubating them for 4 days after crushing in a solution of 1.5 mmol/l $ZnCl_2$ and 1.5 mmol/l picric acid. J_2 that had hatched from the eggs during incubation were then washed in tap water. Ten µl of the aqueous nematode suspension and 10 µl of a 0.1% (w/v) aqueous aniline blue (Aldrich, Milwaukee, Wisconsin) solution (Premachandran *et al.*, 1988) were pipetted onto a glass microscope slide. The solution was encircled by the cover-slip sealer Glyceel (Hooper, 1986). A cover-slip was then placed onto the Glyceel circle and the slides were incubated for 21 h at room temperature in the dark. Light microscopic examination was performed under a Reichert-Jung Polyvar microscope at x 100-1000 magnification. Photographs were taken on 50 ASA Kodak daylight colour films and black and white negatives developed from the colour positives.

Results

The secretion of an aniline blue-stained strand through the "excretory" pore opening of a *H. schachtii* J_2 is shown in Figure 1A. Out of 56 nematodes tested, 53 (94.6%) secreted strands through the pore opening. The strand lengths could exceed the length of the nematode bodies (Fig. 1B). Figure 1A further shows a darker central core and a lighter peripheral region of the strand. In the original colour micrograph the core stains dark blue and the peripheral region light blue. This differential staining of core and peripheral region could only be seen in freshly secreted strands. They partially decomposed during the experiments (Fig. 1B). The decomposition was apparently restricted to the peripheral region; the central core appears intact (Fig. 1C). The decomposed strands resemble a number of ellipsoid bodies that are arranged along the central core like pearls in a necklace (Fig. 1C).

Discussion

The binding specificity of aniline blue is not well understood. Under certain conditions it can be used as a collagen and connective tissue stain (Mallory, 1900; Crossomon, 1937). Aniline blue has at least a low protein specificity so that it binds preferentially to a proteinaceous core

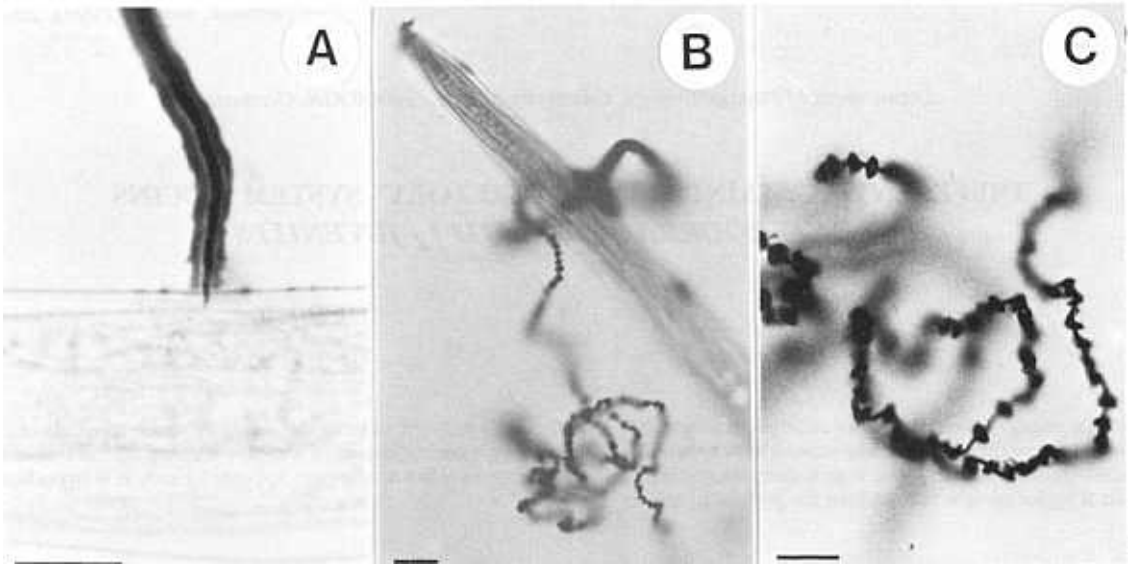

Fig. 1 - Aniline blue-stained "excretory" system mucins of *Heterodera schachtii* J_2-juveniles: A, Secretion through the "excretory" pore; a darker central core and a lighter peripheral region can be differentiated; B, Mucin decomposition increases with incubation time; C, Partially decomposed strand; the central core apparently remained intact, whereas the peripheral region appears decomposed. (scale bar: 10 µm in A and C, 20 µm in B).

region of the secretions of *H. schachtii* J_2. The light blue staining of the peripheral region may then be explained by a lower protein content compared to the core region. A similar differential staining of core and peripheral region can also be seen in spicule secretions of *H. schachtii* males (see Fig. 2D in Aumann and Wyss, 1989).

The formation of strand-like secretions in *H. schachtii* may be caused by the destruction of cell and secretory vesicle membranes by organic solvent components of Glyceel. According to Hooper (1986), Glyceel contains 11.5% (w/v) methanol and 20.4% each of butyl acetate and toluol. The resulting water uptake of the secretory vesicle contents may have caused a manifold volume increase and an extrusion out of the nematode body. The organic solvents may also have caused the decomposition of the peripheral region of the strands (Fig. 1B).

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