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Technique for Axenizing Nematodes[1]

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Many methods have been developed and used for axenizing nematodes (2). For the past 2 years to establish contaminant-free monoxenic cultures we have been using a simple method for axenizing nematodes that to our knowledge has not been described.

The technique is as follows: A glass chromatography column (28 mm i.d. × 300 mm long) with a Teflon stopcock is filled to within a few centimeters of the top with 6-mm-d glass beads. A small piece of screen is inserted in the column above the stopcock to prevent the beads from plugging the orifice of the stopcock. The stopcock is loosened, both ends of the column (or the entire column) are wrapped in aluminum foil, and the column is sterilized by autoclaving.

The column is filled to a few millimeters above the glass beads with an aqueous antibiotic solution prepared with sterile distilled water. We use a solution containing 20 ppm each of streptomycin sulfate and penicillin G, potassium salt.

Xenic nematodes are rinsed three or four times by cycles of suspension of the nematodes in a few milliliters of the above antibiotic solution in a conical 15-ml centrifuge tube, sedimenting the nematodes by brief centrifugation in a clinical centrifuge, drawing off the supernatant, and then resuspending the nematodes in antibiotic solution.

After the last centrifugation the nematodes are transferred in about 0.5 ml of the antibiotic solution with a sterile pasteur pipet to the top of the antibiotic solution in the column. Over a period of 2–4 hours at 25 C the nematodes work their way through the glass beads and have been surface sterilized by the time they collect over the stopcock. The axenic nematodes are then drawn off in 1 or 2 ml of antibiotic solution into a sterile conical 15-ml centrifuge tube and are dispensed with a sterile pipet directly into culture tubes containing alfalfa tissue cultures growing on agar medium slants (1). We have observed no adverse effects from the residual antibiotic on either the nematodes or the plant tissues in the culture tubes.

The purpose of the glass beads in the axenizing column is to break up the vertical convection currents which arise when the nematode suspension is placed on top of the antibiotic solution and the nematodes settle. With glass beads in the column these long convection currents do not form and the contaminating microorganisms are left in suspension at the top of the column rather than being carried to the bottom of the column with the nematodes.

Smaller columns, other materials for disrupting convection currents, and other axenizing solutions might improve this technique for axenizing nematodes.

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