Culture of the Root-Lesion Nematode Pratylenchus vulnus on Carrot Disks

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O'Bannon and Taylor reported that carrot disks on 1% agar were suitable for culturing *Pratylenchus brachyurus* (4). We found that they are suitable also for *Pratylenchus vulnus*, and easier to prepare than the alfalfa tissue we have used in the past (3). Our observations on carrot disks during the last 3 years may be useful to others.

Initially, many of the carrot disks rotted, and a variety of surface-sterilization procedures failed to prevent rotting. Cultures of internal carrot tissue on carrot extract agar or carrot juice agar revealed that apparently healthy carrots had internal infections of *Fusarium solani* (Mart.) Appel and Wr. and three apparently different, but unidentified, species of bacteria. Bacterial infection of apparently healthy vegetables and the stimulation of bacterial soft rot by *Fusarium* have been reported (5, 6). The fungus and bacteria caused a fairly rapid soft rot of the de-topped carrots in plastic bags which we obtained from a store, but little soft rot developed over a 3-month period when carrots with tops still attached were purchased. Carrots with tops usually have not been stored as long as those in plastic bags. Storage, especially in a plastic container, is favorable for bacterial and fungal growth.

Carrots with tops need not be surface-sterilized. Simply remove the tops, scrub the roots, and allow the excess moisture to evaporate. Then, working in a clean location (we use a laminar flow transfer chamber), pare off the external tissue of a carrot in a spiral pattern, flaming the knife before each contact with the carrot. As a carrot is pared, disks 10-15 mm in thickness are cut with the flamed knife into sterile petri dishes. Five disks are later transferred with flamed forceps to culture jars. This number of disks provides sufficient moisture to eliminate the need for water agar.

A reliable method for initial axenization of nematodes is to allow them to crawl through water agar containing disinfestants (1). An effective disinfestant combination is 130 ppm Aretan® (the formulation containing 3% organic mercury; Plant Protection Ltd., Yalding, Kent, England), and 6000 ppm dihydrostreptomycin sulfate. Extract approximately 300,000 nematodes from old cultures (or lower numbers from field material) and concentrate them in 2 ml of water. Prepare and autoclave 250 ml of 1% water agar and cool to 48-50 C. Then add the concentrated nematode suspension to this agar. Swirl the nematode-agar suspension, pour 3 ml into the centers of sterile petri dishes and allow the agar to solidify. Prepare and autoclave one liter of 1% water agar. When it has cooled to 50 C, add 0.13 gm Aretan and 6 gm dihydrostreptomycin sulfate, and agitate until these chemicals are completely dissolved. Pour this antibiotic medium gently over the solidified nematode-agar suspension in each petri dish until the suspension is covered to a depth of 5 mm. After 36-hr incubation at about 23 C, some 1000 nematodes will have made their way up through the antibiotic medium to the surface. Wash these into a 100-ml beaker using 10 ml of sterile water per petri dish. Add 100 surface-sterilized nematodes to each carrot disk culture using a sterile syringe. No bacterial or fungal growth has resulted when lots of 100 of these nematodes have been added to Difco® brain-heart infusion and Czapek-Dox agars. At 23 C *P. vulnus* increases one thousandfold within 3-4 months after inoculation. After 4 months carrot disks become exhausted, or dry. Transfer of 2-month-old cultures to 15 C will retard nematode development and lengthen culture life (3). *P. vulnus* feeds in any tissue in the disk, causing browning of the tissue, which remains firm. Symptoms are different if intrinsic microflora develop in any of the disks. First, a definite purple band appears around the stele. Later the surrounding tissue turns brown, and a soft rot accompanied by leaking develops. Bottles containing disks with these symptoms can be discarded. If such symptoms do not

Received for publication 10 November 1972.

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develop, the intrinsic microflora are either absent or, more likely, present at a very low titer. If they are present at a low level, they will not cause carrots to rot before the nematodes have increased, and they can be eliminated from the nematodes by surface-sterilization.

Pratylenchus vulnus was originally obtained from around *Juglans hindsii* Jepson rootstock in a walnut orchard, and has been cultured 7 years in the laboratory. The first 4 years, the culture medium was a mixture of callus and differentiated alfalfa tissue (3). The last 3 years the medium was carrot disks. In 1971, nematodes with this history increased readily on *J. hindsii* and other fruit and nut tree rootstocks. There is no evidence that the 7 years of culture away from usual hosts has altered this nematode's ability to reproduce on those hosts. Previously, Högger (2) had reported no difference in infectivity on potato of *P. penetrans* from potato in the field, from winter vetch in the greenhouse or from alfalfa tissue culture in the laboratory.

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