

Carrot Callus Tissue for Culture of Endoparasitic Nematodes¹

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Researchers have long sought methods of aseptically cultivating plant-parasitic nematodes. Gnotobiotic culturing of nematodes has been reported in the literature over the years (10,12), but these techniques generally have been considered difficult and time consuming. Recently, however, the three most widely used methods for culturing migratory endoparasitic nematodes—alfalfa callus (1,4,7), root explants (5), and carrot discs (6)—have been published as teaching tools (2,3,9), thus illustrating more practical approaches to gnotobiotic culturing. We present in this paper a supplement to existing techniques. This new method uses carrot callus tissue as the nutrient support for migratory endoparasitic nematodes.

Callus was obtained from 4-week-old surface-sterilized carrot (*Daucus carota* L.) discs (2). Excised callus was transferred to petri plates containing Gamborg's B5 medium (GIBCO, Grand Island, New York) plus 0.1 mg/liter 2,4-dichlorophenoxyacetic acid, 12.5 mg/liter gentamycin sulfate, and 1.5% agar noble. The callus cultures were incubated at 28 C for 6–8 weeks, after which time calli that increased in volume were transferred to fresh plates. Successive transfers and selections were then made to obtain callus cultures that grew rapidly, were friable, and remained in contact with the agar surface.

After the volume of established parent

cultures increased approximately $5 \times$ (4–6 weeks), cultures were subdivided to generate additional callus cultures. The daughter cultures were then inoculated with the following nematodes: *Pratylenchus agilis* Thorne and Malek, *P. scribneri* Steiner (in Sherbakoff and Stanley); *P. brachyurus* (Godfrey) Filipjev; *Radopholus similis* (Cobb) Thorne; and *R. citrophilus* Huettel, Dickson and Kaplan. Nematodes for inoculation were extracted from corn root explant cultures (3) for 18 hours using a modified Baermann technique (8). Extracted nematodes were surface sterilized as described by Huettel and Rebois (3). Culture plates containing ca. 1.5 g callus growth, five replicates per nematode species, were inoculated with ca. 100 nematodes of mixed life stages and incubated at 28 C for 60 days. Two 1.0-g callus growth plates per *Pratylenchus* sp. were incubated for 120 days.

After incubation, the weight of wet callus was determined. Nematodes (mixed life stages and eggs) were then extracted from the wet callus by transferring each callus to a flask containing 30 ml water and agitating for 1 hour at 300 rpm. After agitation, water containing the callus and nematodes was collected on a 25.4- μ m-pore screen and transferred to a 50-ml beaker. The transferred material was gently boiled for 2 minutes in 0.5% cotton blue in lactic acid:glycerin:water (1:1:1). The stained callus and nematodes were decanted onto a 25.4- μ m-pore screen, rinsed with water, and collected in a graduated cylinder. Three 1-ml aliquots were removed from the sample and counted in a Hawksley counting slide on an inverted microscope.

The number of eggs and nematodes extracted from the 60-day-old callus cultures are listed in Table 1. The greatest number of nematodes was recovered from the *P. scribneri* cultures, which yielded a greater

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¹ Mention of a trade name, warranty, proprietary product, or vendor does not constitute a guarantee of a product and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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TABLE 1. Nematodes (mixed life stages) and egg numbers from 60-day-old and 120-day-old carrot callus cultures.

Species	60-day-old cultures†			120-day-old cultures‡		
	Callus wet weight (g)	Eggs	Nematodes	Callus wet weight (g)	Eggs	Nematodes
<i>Pratylenchus agilis</i>	2.60	59	250	0.54	1,010	3,375
<i>Pratylenchus brachyurus</i>	2.90	144	209	1.35	5,041	8,357
<i>Pratylenchus scribneri</i>	4.12	133	1,155	1.39	1,050	6,354
<i>Radopholus citrophilus</i>	4.29	105	696			
<i>Radopholus similis</i>	3.26	25	149			

† Mean of five replications each; initial inoculum density was 100 nematodes per replicate.

‡ Mean of two replications; initial inoculum density was 100 nematodes per replicate.

than 10-fold increase. A 7-fold increase in numbers of nematodes was observed from *R. citrophilus*, and a 2-fold increase was observed for *P. brachyurus* and *P. agilis*. The lowest recovery was from the callus with *R. similis* which only slightly increased from the initial inoculum levels.

The number of nematodes extracted from callus after 120 days indicates the potential yields using this method (Table 1). Yields of more than 4,000 nematodes per gram of wet callus were observed for the three *Pratylenchus* spp.

These results indicate that the carrot callus method is suitable for culturing the nematodes studied, with the possible exception of *R. similis*. We have had success with *R. similis* in other experiments (unpubl.); however, only one temperature was used for cultivation of the nematodes in this study. It has been demonstrated in the literature that optimum temperatures must be established for maximum nematode reproduction (11). Therefore, lower temperatures could increase the cultivation potential of this nematode.

The carrot callus method is easy to use and produces high numbers of nematodes. Cultures can remain viable for up to 5 months, and successive cultures are easily generated. Once the carrot callus is established, the growths can be subdivided into many daughter plates, thus eliminating the need to start with new plant material as in alfalfa callus cultures. Also, once the nematodes are established on the callus, they can be transferred directly in a small infested piece of callus to a new callus plate. This

eliminates having to reesterilize the nematodes during each transfer. These data indicate the suitability of using carrot callus as a convenient method of maintaining migrating endoparasitic nematode populations in gnotobiotic cultures.

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