GNOTOBIOTIC CULTURE OF *PRATYLENCHUS SCRIBNERI* ON CARROT DISCS

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ABSTRACT

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Pratylenchus scribneri obtained from a field population was cultured on 'Williams 79' soybean at 32°C in a growth chamber. Nematodes were surface sterilized using solutions of streptomycin sulfate and either $HgCl_2$ or technical grade Ceresan Nass. Effectiveness of the disinfectants was evaluated by plating surface-sterilized nematodes on potato dextrose agar and by incubating in beef nutrient broth for 7 days at 29°C. *P. scribneri* were axenic and remained viable after soaking in a disinfectant containing 100 ppm $HgCl_2$ and 1000 ppm streptomycin sulfate for 2 min followed by two rinses with sterile distilled water. Nematodes were surface sterilized following migration through agar containing 5 ppm $HgCl_2$ and 10 ppm streptomycin sulfate, but axenization by migration through agar containing 5 ppm Ceresan Nass and 10 ppm streptomycin sulfate was extremely toxic to *P. scribneri*. Viable axenic nematodes obtained after soaking and migration in streptomycin sulfate and $HgCl_2$ were injected into fresh carrot discs and incubated at 29°C. At 70 days, population increases resulting from these axenizing techniques were not significantly different at $P = 0.10$. Up to 771,000 nematodes per culture were obtained after carrot discs were inoculated with approximately 100 treated nematodes.

Additional key words: mercuric chloride, Ceresan Nass, streptomycin sulfate, sterile culture, lesion nematode.

RESUMEN

Lawn, D. A., y G. R. Noel. 1986. Cultivo gnotobiótico de *Pratylenchus scribneri* en discos de zanahoria fresca. Nematrópica 16:45-51.

Pratylenchus scibneri obtenido de una población de campo fue cultivado sobre soja 'Williams 79' a 32°C en una cámara de crecimiento. Los nemátodos fueron esterilizados en la superficie externa utilizando soluciones de sulfato de estreptomicina con $HgCl_2$ o con Ceresan Nass grado técnico. La efectivadad de los desinfectantes fue evaluada por la técnica de places colcando los nemátodos esterlizados superficialmente sobre agar de dextrosa de papa y por incubación en un caldo de nutrientes de carne por 7 dias a 29°C. *P. scribneri* permaneció axénico y viable después de remojo en desinfectante conteniendo 100 ppm de $HgCl_2$ y 1000 ppm de sulfato de estreptomicina por 2 minutos seguidos de dos enjuages con agua destilada estéril. Los nemátodos fueron esterilizados en la superficie después de migrar a través de agar conteniendo 5 ppm $HgCl_2$ y 10 ppm de sulfato de

estreptomicina, sin embargo axenización por migración a través de agar conteniendo 5 ppm de Ceresan Nass y 10 ppm de sulfato de estreptomicina resultó extremadamente tóxica para *P. scribneri*. Nemátodos viables y axénicos obtenidos por remojo y migración en sulfato de estreptomicina y $HgCl_2$ fueron inyectados en discos de zanahoria fresca e incubados a 29°C. A los 70 días, los incrementos de población resultantes de estas técnicas de axenización no fueron significativamente diferentes a P=0.10. Hasta 771,000 nemátodos por cultivo fueron obtenidos después de inocular discos de zanahoria con aproximadamente 100 nemátodos tratados.

Palabras claves adicionales: Ceresan Nass, nemátodo lesionador, cloruro mercúrico, cultivo esterilizado, sulfato de estreptomicina.

INTRODUCTION

Nematode culture on potted host plants requires daily maintenance of the host and typically provides a nematode source contaminated with numerous soil-inhabiting microorganisms. Culturing the thermophylic nematode *Pratylenchus scribneri* Steiner at the optimum reproductive temperature of 31 to 34°C (1) requires the use of a growth chamber or temperature tank. The cost and space required is reduced by rearing certain *Pratylenchus* spp. on carrot (*Daucus carota* L.) discs (7,9).

In earlier reports of gnotobiotic nematode culture, disinfectants such as Aretan® (Bayer AG, Leverkusen, Federal Republic of Germany) that contained a low percentage of organic mercury were effective, but are no longer easily obtainable. Technical grade Ceresan Nass® (Bayer AG, Leverkusen, Federal Republic of Germany), a methoxyethylmercury chloride compound, and mercuric chloride ($HgCl_2$) are potentially more toxic than Aretan since they contain 68.0% organic and 73.9% inorganic mercury, respectively. Surface sterilization of *Aphelenchoides* and *Radopholus* sp. with various concentrations of $HgCl_2$ and several rinses of sterile distilled water have been reported (2,4,5,8). The agar migration method with Aretan was successful with *Paratrichodorus christiei* (Allen) Siddiqi and *Pratylenchus vulnus* Allen and Jensen (6,7). Since Ceresan Nass and $HgCl_2$ are available, evaluation of their toxicity and efficacy for surface sterilization of previously untested nematode species was needed. In this study we describe two methods for producing axenic carrot disc cultures of *P. scribneri* using $HgCl_2$ and streptomycin sulfate as surface sterilants and report on the toxicity of Ceresan Nass to *P. scribneri* when using nematode migration through agar.

MATERIALS AND METHODS

A field population of *P. scribneri* obtained in Illinois was cultured on 'Williams 79' soybean [*Glycine max* (L.) Merr.] in a growth chamber at 32°C. Following mist chamber extraction (10), individual *P. scribneri*

were removed from the nematode suspension using a nylon nematode pick. This monospecific population was reared on carrot discs for 2 mo, and then used as a nematode source for the two axenizing techniques studied.

Agar migration: Approximately 60,000 *P. scribneri* were concentrated into 2 ml of water and added to 50 ml of 1% water agar cooled to 40-45°C. The nematode-agar suspension was distributed evenly into 10 sterile petri dishes. Concentrations of either 5 ppm $HgCl_2$ or 5 ppm Ceresan Nass were mixed with 10 ppm streptomycin sulfate and added to additional 1% water agar cooled to 45°C. The amended water agar was then poured onto the solidified nematode-agar suspension to a depth of 5 mm in each petri dish. These were incubated at 29°C for 36 hr. Nematodes which had migrated to the agar surface were rinsed off the agar with 10 ml of sterile distilled water, counted, and concentrated into 0.5 ml prior to injection into carrot discs. Nine replicates of 10 petri dishes each were conducted.

Soaking: One hundred *P. scribneri* were concentrated in 0.5 ml of water in a test tube to which 5 ml of disinfectant containing 100 ppm $HgCl_2$ and 1000 ppm streptomycin sulfate was added. The contents were agitated, allowed to settle for 2 min and centrifuged at 1000 relative centrifugal force for 30 sec. The disinfectant was decanted immediately to a 0.5 ml volume and the nematodes were rinsed twice by centrifuging aseptically with 5 ml of sterile distilled water. The supernatant was decanted so that a 0.5 ml volume remained for injection into the carrot discs. The soaking protocol was repeated nine times.

When determining the mortality rate of *P. scribneri* using these axenizing techniques, we assumed that a dead nematode or one unable to penetrate carrot tissue would lay outstretched or in an open "c" form with no movement (3). Effectiveness of these axenizing techniques was determined by incubating the treated nematodes on potato dextrose agar (PDA) and in beef nutrient broth (BNB) for 7 days at 29°C.

Carrot disc culture: Fresh carrots with attached tops were washed and peeled under aseptic conditions. Discs were cut approximately 25 to 30 mm in length and a 5 mm indentation was cut in the stele without removing the tissue. Approximately 100 surface sterilized *P. scribneri* were injected under this tissue with a sterile, 20 gauge syringe. Either four or five nematode-inoculated discs were maintained in previously autoclaved 150 ml Fleakers® (Corning Glass Works, Corning, NY, USA) incubated at 29°C. Population increases were determined after 70 days. Nematodes were extracted by slicing the carrot discs into 5 mm-thick cross sections and incubating the sections in a mist chamber at 18°C for 14 days. Mean population differences between the two axenizing

techniques were compared using least square means and Student's t-test of $\log_{10}$ transformed and nontransformed data.

RESULTS AND DISCUSSION

Pratylenchus scribneri survived in the disinfectant containing 100 ppm $HgCl_2$ and 1000 ppm streptomycin sulfate for up to 90 min, however, after 15 min nematode activity decreased. What effects this apparent toxicity had on the nematode infectivity and reproductive capacity could not be determined from this study. Treatment of *P. scribneri* for a minimum of 2 min in a 100 ppm $HgCl_2$ plus 1000 ppm streptomycin sulfate solution followed by two rinses with sterile distilled water was sufficient to inhibit growth of secondary microorganisms when plated on PDA or incubated in BNB.

Table 1. Populations of *Pratylenchus scribneri* recovered following treatment with disinfestants containing $HgCl_2$ and streptomycin sulfate (SS) and incubation in carrot discs for 70 days.

Axenizing technique	Number inoculated	Number recovered[z] (x1000)	Population increase[z] (x100)
Agar migration:	68	35	5
	88	51	6
5 ppm $HgCl_2$ + 10 ppm SS	80	254	32
	113	771	68
	220	477	24
	85	131	15
	50	617	123
	90	97	11
	95	431	43
2 min soak:	100	98	10
	100	226	23
100 ppm $HgCl_2$ + 1000 ppm SS	100	53	5
	100	338	34
	100	221	22
	100	47	5
	100	694	69
	100	408	41
	100	105	10

[z]No significant differences between axenizing techniques in either number recovered or population increase.

The average number of nematodes recovered after migration through agar containing $HgCl_2$ varied from 50 to 200 per trial (Table 1). This represents only those active individuals because it was assumed that nematode death could have occurred following their upward migration to the agar surface. No microorganisms were associated with these nematodes on PDA or in BNB. Variability in numbers of living nematodes recovered from the 9 trials using this technique may have resulted from slight variations in depth of agar among petri dishes through which the nematodes migrated. Nematode migration might be enhanced by incubating the plates at temperatures greater than 29°C. Ceresan Nass was highly toxic to nematodes migrating through agar since less than 10 viable *P. scribneri* were recovered per trial. Because of this low recovery rate, additional attempts to surface sterilize *P. scribneri* using Ceresan Nass were discontinued. Since the concentration of mercury in Ceresan Nass and $HgCl_2$ was similar, an ingredient other than mercury in Ceresan Nass was presumed responsible for the toxicity to *P. scribneri.*

Numbers of *P. scribneri* recovered and their population increases were not different ($P=0.10$) when comparing the two $HgCl_2$ and streptomycin sulfate axenizing techniques using either $\log_{10}$ transformed or nontransformed data (Table 1). The low water temperature in the mist chamber probably restricted nematode migration from the discs and more nematodes would have been extracted in less time if the water temperature had been higher. Greater increases also would be expected if discs were incubated at 35°C which is the optimum temperature for *P. scribneri.* Large numbers of second-stage juveniles began to emerge after 7 days as eggs hatched, but were not counted in the population.

Following nematode inoculation, healthy carrot discs were characterized by white callus growth. Sandstedt and Schuster (11) observed greater callus growth at the radical end of carrot discs when inoculated with *Meloidogyne incognita* (Kofoid & White) Chitwood, and suggested that this was partly due to enhanced nematode nutrition at the radical end. A similar growth pattern of callus tissue was observed in this study, but discs were placed randomly in the fleakers resulting in both apical and radical ends of discs being inoculated, which may explain partly why nematode increases varied between replications. Variability in the life stages of the initial nematode inoculum also may account for the differences. With time, the white callus growth changed to a brown-bronze color. Some carrot tissue began degrading after 70 days. The carrots used for establishing these cultures may have had internal bacterial contamination. We have maintained other cultures more than 90 days at 25°C and have observed no contamination, indicating that a temperature relationship may occur.

Nematodes were observed migrating from discs as they moved *en masse* up the jar walls. An efficient method of transferring nematodes to fresh carrot discs was to cut a 5-mm slice from a healthy callus-covered disc, quarter the disc, and place it atop fresh discs for 3 days. In 3 days a sufficient number of *Pratylenchus* was transferred with minimal contamination.

We have maintained monoxenic cultures of *P. scribneri* for two yr. The techniques developed and described in this paper have provided large numbers of axenic nematodes for other studies, and should provide researchers studying other *Pratylenchus* species a method for producing monoxenic cultures.

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