

# RESEARCH NOTES

Journal of Nematology 19(2):255-256. 1987.  
© The Society of Nematologists 1987.

## A Semi-selective Medium for the Isolation of *Paecilomyces lilacinus* from Soil<sup>1</sup>

D. J. MITCHELL,<sup>2</sup> M. E. KANNWISCHER-MITCHELL,<sup>3</sup> AND D. W. DICKSON<sup>3</sup>

*Keywords:* biological management, fungal isolation, root-knot nematode, cyst nematode, fungal population dynamics.

*Paecilomyces lilacinus* (Thom) Sampson has been observed to parasitize eggs, juveniles within eggs, and adult females of root-knot nematodes (*Meloidogyne* spp.) and eggs and females of cyst nematodes (species of *Globodera* and *Heterodera*) (2-5). Jatala et al. (3) reported the use of *P. lilacinus* in the biological management of *M. incognita acrita* and *G. pallida* in Peru, and interest has developed subsequently in the use of this fungus in many regions of the world.

The medium described here was developed to study the survival and population dynamics of *P. lilacinus* in soil. It is useful also in the detection of nematodes infected by *P. lilacinus*. Although *P. lilacinus* can be cultured readily on various standard fungal growth media, these media are not suitable for isolation of this fungus from soil because its growth often is inhibited or its presence is obscured by the rapid growth of bacteria or other fungi. A medium developed by Steiner and Watson (6) is useful for the isolation of various fungi from soil and from plant or nematode tissue; the medium contains streptomycin sulfate and a surfactant, Tergitol NP10, in potato dextrose agar (PDA). Although growth of

many bacteria is inhibited by the antibiotic, and the growth of fungi is slowed by the presence of Tergitol NP10, this medium did not sufficiently inhibit growth of some commonly occurring soil-borne fungi. For example, species of *Rhizopus* and *Trichoderma* may grow over and obscure the more slowly growing colonies of *P. lilacinus*. Therefore, this medium had to be amended to favor the isolation of *P. lilacinus*.

*Paecilomyces lilacinus* is more tolerant of higher salt concentrations than are many other fungi (1), and the incorporation of 1-3% of NaCl into the medium reduced the number of fungal species isolated without inhibiting growth or sporulation of *P. lilacinus*. Incorporation of pentachloronitrobenzene and benomyl into the medium inhibited *Rhizopus* spp. and *Trichoderma* spp., respectively, without affecting the detection of *P. lilacinus*. Chlortetracycline was included in the medium to further suppress the growth of bacteria.

The medium was prepared by combining 10 g NaCl, 50 mg pentachloronitrobenzene (PCNB; Terraclor, 75% a.i., Olin Mathieson Chemical Corp., Little Rock, Arkansas), 50 mg benomyl (Benlate, 50% a.i., E. I. duPont de Nemours & Co., Wilmington, Delaware), 39 g potato dextrose agar (Difco Laboratories, Inc., Detroit, Michigan) and deionized water to bring the volume to 1 liter. After the medium was autoclaved for 15 minutes at  $1.01 \times 10^5$  N/m<sup>2</sup> (15 psi) and cooled to 45-50 C, 100 mg of streptomycin sulfate (Sigma Chemical Co., St. Louis, Missouri), 50 mg of chlortetracycline hydrochloride (Sigma Chemical Co.), and 1 ml of Tergitol NP10 (Sigma Chemical Co.) were added.

Received for publication 19 May 1986.

<sup>1</sup> Florida Agricultural Experiment Stations Journal Series, No. 7561.

<sup>2</sup> Professor, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

<sup>3</sup> Postdoctoral Associate and Professor, Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

The authors thank P. Rayside and T. Hewlett for technical assistance.

The medium can be dispensed immediately into petri dishes; however, for the quantification of *P. lilacinus* in soil, a dilution-plating procedure gives the best results. A serial dilution of soil, starting with 1–5 g of soil, is made in sterile, deionized water. The final dilution will depend on the population density of the fungus; therefore, it may be necessary to try a range of dilutions initially to establish the proper dilution to use. For example, in naturally infested field soil, a dilution of 1:2,000 (soil : water) may be a good starting point, whereas dilutions of up to 1:15,000 (soil : water) have been used in artificially infested soil.

One milliliter of soil water suspension is pipetted into an empty sterile petri dish (10 dishes per sample), and approximately 15 ml of cooled medium is added to each petri dish. The dishes are swirled gently to distribute the sample in the medium. Petri dishes should be incubated at 25–27 C with 12 hours of light for 7–10 days. The color of *P. lilacinus* colonies on this medium generally ranges from lavender to dark purple, and, depending on the isolate of the fungus, the surface may be fluffy or smooth and wet in appearance.

This medium has been used successfully for more than 2 years to quantify soil populations of *P. lilacinus* in greenhouse and field experiments. Estimations of population densities based on the use of this medium have ranged from less than 200 prop-

agules per gram of natural field soil to more than  $2 \times 10^6$  propagules per gram of soil in field plots artificially infested with *P. lilacinus* grown on autoclaved wheat seeds. Quantitation of soil population densities of fungi potentially antagonistic to phytopathogenic nematodes is important in the evaluation of the effectiveness and longevity of treatments. Selective media, such as that described here, can improve the detection of these fungi from soil or infected nematodes.

#### LITERATURE CITED

1. Domsch, K. H., W. Gams, and T. H. Anderson. 1980. Compendium of soil fungi, vol. 1. New York: Academic Press.
2. Gintis, B. O., G. Morgan-Jones, and R. Rodríguez-Kábana. 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. *Nematropica* 13:181–200.
3. Jatala, P., R. Kaltenbach, and M. Bocangel. 1979. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. *Journal of Nematology* 11:303 (Abstr.).
4. Jatala, P., R. Kaltenbach, M. Bocangel, A. J. Devaux, and R. Campos. 1980. Field application of *Paecilomyces lilacinus* for controlling *Meloidogyne incognita* on potatoes. *Journal of Nematology* 12:226 (Abstr.).
5. Morgan-Jones, G., J. F. White, and R. Rodríguez-Kábana. 1984. Fungal parasites of *Meloidogyne incognita* in an Alabama soybean field soil. *Nematropica* 14:93–96.
6. Steiner, G. W., and R. D. Watson. 1965. Use of surfactants in the soil dilution and plate count method. *Phytopathology* 55:728–730.