COMPARISON OF THE EFFICACY OF *PASTEURIA PENETRANS* ENDOSPORES PRODUCED *INVIVO* AND *INVITRO* FOR THE CONTROL OF *MELOIDOGYNE ARENARIA*.

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Abstract

Pasteuria spp., obligate parasites of most plant parasitic nematodes, are promising biological control agents. *P. penetrans* produces spores that adhere to the cuticle of root-knot nematodes. When high densities of these spores are present in the soil the rate of nematode root infection can be greatly reduced. Nematodes attached with spores that are able to enter root system are parasitized by these bacteria, which grow vegetatively inside the body and inhibit egg production. The bacteria then sporulate, filling the female body with spores, which are eventually released in to the soil. Mass production of these bacteria is necessary for it to be an economical agricultural product. Pasteuria Bioscience, LLC has recently developed culture techniques for production of *P. penetrans* endospores in vitro, in defined media. Determining the efficacy of these *in vitro*-produced spores in control of root-knot nematodes is the first step in developing *P. penetrans* into a viable biological control product.

Introduction

Plant parasitic nematodes cause crop losses to agriculture estimated at US$ 78 billion worldwide. In the United States increased environmental concerns has caused the eventual removal of Methyl Bromide from the market and rapid reduction in the number of remaining nematicides available to growers. Many sectors of commercial and non-commercial agriculture have no chemical control products available. Therefore the need for more nematode control products has increase with few chemical companies willing to invest the time and money to create and bring to market new products to fill this void.

Laboratory and field tests have demonstrated the efficacy of *Pasteuria* sp. for controlling nematode populations below economic threshold levels (Chen and Dickson, 1998). *P. penetrans* is currently produced *in-vivo* and used for root-knot nematode control in Japanese vegetable production and at Disney World EPCOT Center in an agricultural display. The bacteria are produced, *in vivo*, by attaching spores to large number of root-knot juveniles that are inoculated onto a suitable host plant. Plant root systems are later harvested, dried and ground to form a spore-laden root powder. The cost of producing *P. penetrans* using this method is estimated to be between $6000- $15000 per acre. *In-vitro* cultivation of this bacterium in defined media would allow for mass production of spores at an economically viable cost. Initial attempts at *in-vitro* culture (Reise et al., 1988; Reise et al., 1991) (Bishop and Ellar, 1991) were not successful, and investment and interest in development of *Pasteuria* spp. as a nematode control product was greatly diminished. Pasteuria Bioscience and its predecessor company, Entomos, Inc., have been developing *in vitro* culture methods for *P.penetrans* since the year 2000. Complex media have now been developed that support growth and sporulation of *P. penetrans.* The efficacy of these spores, and their rate of attachment and infection, were compared to that of spores produced *in vivo* in a laboratory experiment.

Materials and Methods

The *Pasteuria penetrans* isolate used in this study was extracted from *Meloidogyne arenaria* females collected from the roots of a peanut plant in a field in Alachua County, Florida, USA. *M. arenaria* and *P. penetrans* cultures were grown on tomatoes (CV Rutgers) in a greenhouse. Spore suspensions were produced by harvesting tomato roots containing 60-day-old *P. penetrans* infected females. *In-vitro* spores were harvested from several culture experiments that had produced mature spores. Spore-containing cultures were rinsed from dishes, centrifuged and washed to remove growth media. Both *in-vitro* and *in-vivo* spores were sonicated for 3 minutes to remove the outer coatings of the spores and allow for increased attachment. Spore concentrations for both *in vivo* and *in vitro* preparations were determined with the use of a hemacytometer.

The evaluation was carried out in the laboratory of Dr. William Crow, Extension Nematologist at the University of Florida. Treatments included a control of healthy plants without nematodes or spores, a control with nematodes but without spores, and soil inoculated with nematodes and in vivo or in vitro spores. Treatments were replicated five times. The soil used was an Arendondo fine sand (95, 2.5, 2.5: sand, silt and clay). *In vivo* and in vitro spores were inoculated into soil to produce a density of 10^5 endospores/cc of soil. Soil with each type of spore was thoroughly mixed and allowed to air-dry on a tray for 24 hours. Twenty-five cc's of *in vivo* and *in vitro* spore-treated soil were placed into 50 ml centrifuge tubes. *M.* arenaria juveniles, 1-5 days old, collected from greenhouse culture were inoculated into each treated tube, 500 juveniles/tube. Tomato seedlings, 3-4 weeks old, were transplanted into each tube. Plants were incubated at 30^{o}C in lighted incubators. After 24 days, root systems of plants were washed clean and data collected. The number of root-knot galls, egg masses and eggs per root system was recorded. Females were extracted from the root systems and the percentage of females infected with *P. penetrans* was determined as shown in Table 1.

Results

Although an 87% reduction in total nematode eggs was observed on plants treated with in-vitro spores compared to untreated controls, there were no statistically significant differences in galling, egg masses, or number of eggs between treatments in tubes inoculated with nematodes. High variation among replicates in most treatments is attributed to the experimental design using a low volume of soil. The low volume resulted in a small distance necessary for nematodes to move to reach root tips, therefore decreasing the amount of time nematodes were exposed to spores in the soil.

There was no significant difference between spores produced *in-vitro* and *in-vivo* in percentage of females infected. Spores produced *in-vitro* are therefore similar to those produced *in-vivo* in attachment and infectivity of root-knot nematodes. Further replications of this test in larger pots will be necessary to fully demonstrate the efficacy of spores produced *in-vitro*. Presently Pasteuria Bioscience is working on scale-up fermentation of *P. penetrans* to allow for larger scale greenhouse and microplot tests .

Table 1. Results of efficacy of in vitro spores compared with in vivo spores in control of root-knot nematodes on tomato. Numbers are averages of five replicates.

	Galls		Egg masses		Eggs		%infected	
Treatments	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control no nemas	0	0	0	0	0	0	0	0
Control no spores	16	8.5	12.6	18.2	2118.6	3364.1	0	0
In vivo spores	18.4	13.1	12	14.5	944.4	1123.8	16	26.1
In vitro spores	13.6	15.4	6.8	10.9	273.9	466.6	13.7	17.0

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