EFFICACY OF IN-VITRO *PASTEURIA SPP*. PARASITIZING TWO NEMATODE SPECIES.

T. E. Hewlett*, Pasteuria Bioscience Inc., S. Griswold , J. Waters and K. Smith. Alachua, FL, www.pasteuriabio.com.

Introduction

Pasteuria species have long been identified as obligately parasitic bacteria of many nematode species. Fermentation techniques developed by Pasteuria Bioscience Inc. have made it possible to produce vegetative cells and spores of several P*asteuria* species in -vitro. Past studies of the rate of *Pasteuria* species germination and growth inside nematodes were hindered by the mistaken identification of mycelial structures as vegetative cells. *Pasteuria* cells are difficult to recognize during observations in these types of studies as they involve crushing the nematode body on a wet mount slide with a cover slip. Nematode body content hinders observation of cells and early mycelial structure. Due to rapid vegetative growth, *Pasteuria* cells that originate inside of nematodes are possible to observe when nematodes are crushed in Pasteuria Bioscience proprietary growth media.

Methods

The rate of infection and sporulation of two species, *Pasteuria penetrans*, which parasitizes root-knot nematodes and (Candidatus) *P. usgae*, parasite of sting nematodes, were tested in the laboratory. Endospores of these two species were produced in-vitro and attached to nematodes using a centrifuge technique. Spore encumbered nematodes were placed in an aerated water bath or in clean moist soil at 23° C. At two-day intervals nematode samples were collected (for a total of 8 days), surface sterilized and crushed into 6 well tissue culture plates with growth media. Samples were immediately observed and scored for presence of , mycelial balls, thalli and spores. Observations were made for presents of cells after 3 hours.

Results and Conclusion

Cells were present inside bodies of both nematode species at 2 days, cells and mycelia balls were present at 4 days and thalli and some spores were present at day 6 and 8. Initial studies of the life cycle of *P. penetrans* (in-vivo) spores in root knot nematodes reported germination occurring at 6 days after attachment. It has also been reported for *Pasteuria penetrans* that germination will only take place after the nematode sets up a root feeding site. Our results show that in-vitro spores germinate soon after attachment is complete. In-vivo, cells grow rapidly and can sporulate in root-knot and sting juveniles within 8 days. Post- plant application of *Pasteuria* spores in certain conditions may therefore have greater efficacy than at- plant applications. Nematodes infected with *Pasteuria* and without an available host plant may be parasitized and die before crops are planted.