

PASTEURIA FOR NEMATODE CONTROL: DEVELOPMENT OF A COMMERCIAL PRODUCTION PROCESS

Kelly S. Smith, Thomas E. Hewlett, and Susan Griswold, Pasteuria Bioscience, LLC, Alachua, FL

Bacteria of the genus *Pasteuria* have long been recognized as promising biological control agents for plant-parasitic nematodes (Chen, 1998; Bishop and Ellar, 1991; Sayre, 1980). They are known for their ability to disrupt reproduction in their nematode hosts (Ahmad and Gowen, 1991) and have been shown to reduce root-knot nematode damage below economic threshold levels and to improve crop yields (Brown, et al, 1985).

Pasteuria endospores are the infective agent applied to crops for nematode control; they are very desirable as nematode control products due to their inherent resistance to heat, drying and mechanical shearing. The major impediment to their use as a commercial nematode control product has always been the inability of researchers to grow them in the absence of a nematode host, thus precluding the use of large-scale, low-cost production methods (Chen, 1998; Bishop and Ellar, 1991).

Pasteuria Bioscience was formed to scale-up and commercialize technology for in-vitro culture of *Pasteuria* spp. for nematode control. This technology was first developed at Entomos, Inc. and reported at this meeting in 2002. Since that time our effort has focused on development of a complex medium formulation, and discovery of fermentation conditions, that allow sustained growth and sporulation of *Pasteuria*. In addition, we have continued to pursue methods that are compatible with scale-up and eventual commercial production. Using fractional factorial and central composite experimental designs, we have determined the classes of compounds that are suitable carbon and nitrogen sources for *Pasteuria* spp., as well as vitamins, trace minerals and other fermentation conditions.

Although several types of fermentation processes are commonly used for commercial production processes, submerged (liquid culture) fermentation is commonly used for production of biopesticides such as *Bacillus thuringiensis*, and is well-understood as an economical production method for a huge variety of industrial products (Bailey and Ollis, 1986; Hsu, 2002). We have developed a production process using submerged fermentation, which has allowed us to scale-up from work in tissue-culture plates to benchtop fermenters. Batch and fed-batch production at the benchtop scale will allow efficient translation of production conditions to larger pilot and commercial scale fermenters. Our process models indicate that production will be possible at costs competitive with chemical nematicides, using standard fermentation equipment.

We also have continued to test *Pasteuria* endospores produced in-vitro for nematode control activity (Hewlett, et al 2003) and have found them to be as effective or better than in-vivo endospores in these experiments. In addition, we have begun development work on a fermentation process for *Pasteuria* isolated from Lance nematode, and have demonstrated growth of *Pasteuria* from Cyst and Sting nematode in tissue culture plates.

Pasteuria spp. remain an attractive prospect for a new commercial nematode control agent. Our recent progress on production methods indicates that large-scale production of *Pasteuria* endospores is not only possible, but feasible and cost-effective.

References

- Ahmad, R. and S. R. Gowen.** 1991. Studies on the Infection of *Meloidogyne* spp. with Isolates of *Pasteuria* penetrans. Nematol.Mediterranea **19**:233.
- Bailey, J. E. and D. F. Ollis.** 1986. Biochemical Engineering Fundamentals. McGraw-Hill Publishing Co., New York.
- Bishop, A. H. and D. J. Ellar.** 1991. Attempts to culture *Pasteuria penetrans* *in vitro*. Biocontrol Sci.Tech. **1**:101-114.
- Brown, S. M., J. L. Kepner, and G. C. Smart, Jr.** 1985. Increased Crop Yields Following Application of *Bacillus Penetrans* to Field Plots Infested With *Meloidogyne Incognita*. Soil Biol.Biochem. **17**:483-486.
- Chen, Z. X. and D. W. Dickson.** 1998. Review of *Pasteuria penetrans*: Biology, Ecology, and Biological Control Potential. J.Nematol. **30**:313-340.
- Hewlett, T. E., K. S. Smith, S. T. Griswold, and W. T. Crow.** 2003. Comparison of the efficacy of *Pasteuria penetrans* endospores produced *in vivo* and *in vitro* for the control of *Meloidogyne arenaria*. Proceedings of the Methyl Bromide Alternatives Organization Annual Meeting .
- Hsu, Y.-L. and W.-T. Wu.** 2002. A novel approach for scaling-up a fermentation system. Biochem.Eng.J. **11**:123-130.
- Sayre, R. M.** 1980. Biocontrol: *Bacillus penetrans* and Related Parasites of Nematodes. J.Nematol. **12**:260-269.