Understanding nematode suppressive soils: molecular interactions between *Pasteuria* endospores and the nematode surface coat

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Summary

The knowledge that the plant-parasitic nematode hyperparasite *Pasteuria penetrans* is important in nematode suppressive soils has long been recognised. The ability to mass produce this organism *in vitro* circumvents one of its major constraints. However, successful biological control can only be established if the strains that are deployed can attach to and infect pest nematodes. Currently, in respect to the *Pasteuria* that infects root-knot nematodes (*Meloidogyne* spp), it is thought that collagen-like fibres on the surface of the endospore are interacting with a receptor on the nematode cuticle and that mucin-like molecules play an important role in modulating this process. Here we report that an antibody raised to whole endospores of *P. penetrans* also recognises extracts from endospores of *Bacillus thuringiensis* (Bt)*,* suggesting that Bt can be used as a model for *Pasteuria* endospores. Bioinformatics shows that mucin-like genes identified in *C. elegans* are present in *Globodera pallida*.

Key words: *Pasteuria*, nematode cuticle, surface coat, mucins, suppressive soils

Introduction

Continuous cropping of cereals leads to increases of cereal cyst nematode (*Heterodera avenae*) with associated loss of yield for 3–8 years. However, continued mono-cropping produces increases in crop yields with decreases in nematode numbers (Gair *et al*. 1969). Research has shown nematode suppression is at least in part due to a build-up of microbial parasites. A number of bacteria and fungi have been shown to be responsible for the development of nematode suppressive soils. These can broadly be divided into obligate and facultative microbial parasites (Table 1). Companies have concentrated on developing commercial products by focusing on facultative parasites (e.g. *Arthrobotrys* and *Purpureocillium* spp.) as these are easy to mass produce. Recent progress has been made on culturing the obligate nematode hyperparasite *Pasteuria penetrans* and other *Pasteuria* species. Syngenta Limited have a product, *Clariva* (http://www.syngentacropprotection.com/clariva-complete-beans-seed-treatment?tab=details), based on *Pasteuria* that is being used to control *Heterodera glycines*.
Little work has been done on the interaction between *Pasteuria* and potato cyst nematodes (PCN; *Globodera* spp.) which is a pest problem in both the United Kingdom and India. However, recently an isolate from *Heterodera cajani* has been found to attach to and infect

PCN (Mohan *et al*., 2012). The British Council through their UKIERI program have been supporting work on genomics of *Pasteuria* spp. to explore the mechanism of its attachment to PCN (Srivastava *et al*., 2014). Our working hypothesis is that collagen-like fibrils on the surface of the *Pasteuria* endospore are interacting with a receptor on the infective juvenile cuticle and that the interaction is modulated by mucin-like molecules of the surface coat (Davies, 2009; Davies and Curtis, 2011). In this study we report preliminary experiments and bioinformatic studies to see if there are similarities between the endospores of *Bacillus thuringiensis* and *Pasteuria penetrans* and the identification of mucin-like genes in *Globodera pallida*.

Materials and Methods

Comparison of endospore extracts between Bacillus thuringiensis *and* Pasteuria penetrans

Extracts of endospores from *Pasteuria penetrans* and *Bacillus thuringiensis* were run on SDS-Page gels, electroblotted onto a PVDF membrane and visualised using a polyclonal antibody made to whole spores of *Pasteuria penetrans* following the method of Davies *et al*. (1992).

Identification of mucin-like molecules in Globodera pallida using bioinformatics

A number of mucin-like genes have been identified in *Caenorhabditis elegans* (http://www.medkem.gu.se/mucinbiology/databases/db/Mucin-celegans.htm) and knocking down these genes using an RNAi feeding approach showed that the recognition of the cuticle using fluorescently (FITC)-labelled lectins showed a change in their binding pattern to the adult hermaphrodite cuticle (Davies *et al*., 2009). BLAST searches were undertaken to see which of these mucin-like molecules were also present in *G. pallida*.

Results

Comparison of endospore extracts between Bacillus thuringiensis *and* Pasteuria penetrans

Fig. 1 shows that the antibody raised against whole spores of *Pasteuria penetrans* cross reacts and recognises the endospore extract from *B. thuringiensis* and that the antibody recognised several bands at 250, 100 and 75 kDa in *B. thuringiensis,* whereas it appears to only recognise a single band at 75 kDa in *P. penetrans* spore extract. The pre-immune sera did not recognise any bands from either of the two bacterial endospore extracts although there was evidence of a weak smear present in the *B. thuringiensis* lane.
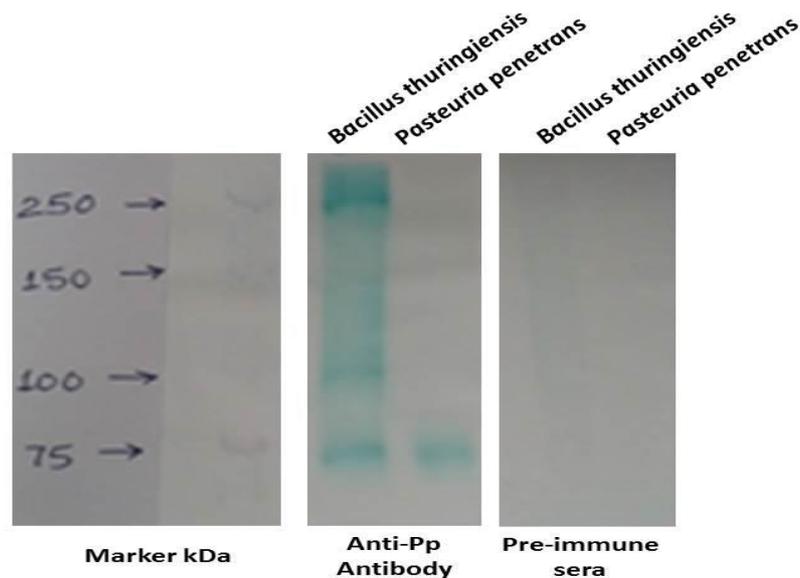
Fig. 1. Western blot of endospore extracts from *Bacillus thuringiensis* and *Pasteuria penetrans* probed with a polyclonal antibody raised to whole spores of *Pasteuria penetrans* and a pre-immune sera.

Identification of mucin-like molecules in Globodera pallida *using bioinformatics*

Of the 13 mucin-like genes present in *C. elegans* over 60% (nine individual top hits) were recognised in *G. pallida* with a Score over 150 and an E-value less than 1.0 e^{-09}. This compares with over 85% (11 individual top hits) having a hit to *M. hapla* with a Score greater than 150. Indeed 77 % of these had E-values less than 1.0 e^{-15} and four of these mucins (K06A9.1, F59A6.3, T19D12.1 and C12D12.1) have E-values of less than 1.0 e^{-20}. Interestingly only seven individual top hits to *M. incognita* had a Score over 150 (data not presented).

Discussion

The successful deployment of *Pasteuria* spp as a biological control agent requires that the endospores attach to the cuticle of the second-stage juvenile as it migrates through the soil towards the plant root. Various isolates of *Pasteuria* exhibit different host ranges from those that are highly specific (Davies, 2009) to those that are more promiscuous (Mohan *et al.*, 2012). Understanding the mechanism of attachment would therefore provide insights into their likelihood to attach to infective juveniles and prohibit their ability to reproduce and hence their likelihood to control the nematode in a given field situation. To date, although we have several genome sequences of plant-parasitic nematodes we only have survey sequences available for *Pasteuria*. Therefore, we have to take advantage of the sequences we do have.

The preliminary work presented here shows that a polyclonal antibody made to whole endospores of *Pasteuria* cross reacts with endospores of *Bacillus thuringiensis*. As much more research has been done on *Bacillus* species than on *Pasteuria* it should be possible to use these closely-related species as models from which it should be possible to generate hypotheses about the form and function of the various components of the endospore and test whether this knowledge can be applied to *Pasteuria*. A similar approach can also be applied to the role of mucin-like genes in *C. elegans* and to see their role, if any, in endospore attachment.

It is widely acknowledged *Pasteuria* species are involved in an arms race (Davies, 2009) and in order for them to be deployed as plant-parasitic nematode control agents both sides of this interaction need to be understood. The use of *Pasteuria* without due concern for the compatibilities between the bacterium and its nematode host will only lead to disappointment.

Acknowledgements

References

Davies K G. 2009. Understanding the interaction between an obligate hyperparasitic bacterium, *Pasteuria penetrans* and its obligate plant parasitic nematode host, *Meloidogyne* spp. *Advances in Parasitology* **68**:211–245.

Davies K G, Gravato-Nobre M, Hodgkin J. 2009. Knock down of mucin like genes changes lectin staining. University of California, Los Angeles: *17th International C. elegans meeting 24-28 June*.

Davies K G, Curtis R H. 2011. Cuticle surface coat of plant-parasitic nematodes. *Annual. Reviews of Phytopathology* **49**:135-156.

Davies, K. G., Robinson, M. P. & Laird, V. 1992. Proteins on the surface of spores of *Pasteuria penetrans* and their involvement in attachment to the cuticle of second-stage juveniles of *Meloidogyne incognita. Journal of Invertebrate Pathology* 59, 18-23.

Gair R, Mathias P L, Harvey P N. 1969. Studies of cereal cyst nematode populations and cereal yields under continuous or intensive culture. *Annals of Applied Biology* **63**:503-512.

Jaffee B A, Zehr E I. 1982. Parasitism of the nematode *Criconemella xenoplax* by the fungus *Hirsutella rhossiliensis*. *Phytopathology* **72**:1378–1381.

Kerry, B.R. and Hirsch, P.R. 2011. Ecology of *Pochonia chlamydosporia* in the rhizosphere at the population, whole organism and molecular scales. In *Biological Control of Plant-Parasitic Nematodes: Building Coherance between Microbial Ecology and Molecular Mechanisms* (Eds Keith Davies & Yitzhak Spiegel) p 171 - 182. Springer.

Mohan S, Mauchline T H, Rowe J, Hirsch P R, Davies K G. 2012. *Pasteuria* endospores from *Heterodera cajani* (Nematoda: Heteroderidae) exhibit inverted attachment and altered germination in cross-infection studies with *Globodera pallida* (Nematoda: Heteroderidae) *FEMS Microbiology Ecology* **79**:675–684.

Sharon, E. Chet, I and Spiegel, Y. 2011. *Trichoderma* as a biological control agent. In *Biological Control of Plant-Parasitic Nematodes: Building Coherance between Microbial*

Ecology and Molecular Mechanisms (Eds Keith Davies & Yitzhak Spiegel) p 183 - 201. Springer.

Srivastava A, Hall A M, Graeme-Cook K, Davies K G. 2014. Exploiting genomics to improve the biological control potential of *Pasteuria* spp., an organism with potential to control plant-parasitic nematodes. *Aspects of Applied Biology* **127**, *Crop Production in Southern Britain: Precision Decisions for Profitable Cropping*, pp. 9–14.

Tunlid, A. and Ahrén D. 2011. Molecular mechanisms of the interaction between nematode-trapping fungi and nematodes: Lessons from genomics. In *Biological Control of Plant-Parasitic Nematodes: Building Coherance between Microbial Ecology and Molecular Mechanisms* (Eds Keith Davies & Yitzhak Spiegel) p 145- 169. Springer.

Table 1. *Examples of facultative and obligate microbial parasites of plant-parasitic nematodes identified as potential biological control agents*

Microbe	Mode of parasitism	Reference
Facultative		
Arthrobotrys spp	Trapping fungi	Tunlid & Ahren (2011)
Pochonia chlamydosporia	Adult females and eggs	Kerry & Hirsch (2011)
Trichoderma spp	egg parasite	Sharon *et al.,* (2011)
Obligate		
Pasteuria spp	Developing juveniles/females	Davies (2009)
Nematophthora gynophila	Adult females	Kerry & Hirsch (2011)
Hirsutella rhossilliensis	Infective juveniles	Jaffee & Zehr (1982)

Table 2. *Bioinformatic search giving the tBLAST n Score and E-value of the mucin-like protein sequences from* Caenorhabditis elegans *against the root-knot nematode* Meloidogyne hapla *and potato cyst nematode* Globodera pallida *using the algorithm at www.nematode.net and searching the transcribed data set*

C. elegans	Meloidogyne hapla		Globodera pallida	
Mucin	Score	E-value	Score	E-value
K06A9.1	265	$1.6\ e^{-22}$	170	$3.9\ e^{-11}$
H02F09.3	202	$1.1\ e^{-15}$	182	$1.9\ e^{-12}$
F59A6.3	284	$9.4\ e^{-25}$	195	$2.2\ e^{-14}$
H43E16.1	214	$5.6\ e^{-17}$	173	$2.1\ e^{-11}$
T19D12.1	279	$1.2\ e^{-23}$	176	$1.6\ e^{-11}$
F53B7.5	213	$1.9\ e^{-16}$	168	$1.5\ e^{-10}$
C12D12.1	303	$1.0\ e^{-26}$	177	$1.1\ e^{-13}$
F35E12.7a	148	$1.1\ e^{-08}$	151	$4.0\ e^{-09}$
F16F9.2	225	$4.0\ e^{-18}$	186	$4.3\ e^{-13}$
C26G2.2	155	$2.5\ e^{-09}$	122	$1.3\ e^{-05}$
LET-653	206	$3.8\ e^{-16}$	145	$2.2\ e^{-08}$
CPG-1	126	$146\ e^{-13}$	135	$1.9\ e^{-07}$
CWP-4	220	$2.4\ e^{-18}$	129	$5.0\ e^{-07}$