Biological Control and *Pasteuria penetrans*:
genomics and molecular biology

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International Nematology Course
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www.rothamsted.bbsrc.ac.uk/ppi/staff/kgd.html
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Structure of talk:

- Introduction and context
- Immunological approach to host specificity
- Genomics and taxonomy
- *In vitro* culture
- Collagen and the mechanism of attachment
- Three types of cuticle variation
The life-cycle of *Pasteuria penetrans* on root-knot nematodes (*Advances in Parasitol. In press*)

- Penetration peg
- Rhizoids
- Rod
- Adhesion to cuticle
- Maturation
- Granular masses
- Sporulation
- Mature endospores
Increase in tomato yield following application of *Pasteuria penetrans* to root-knot infected soils in Ecuador
adapted from Trudgill *et al.*, (2000) *Nematology* 2, 823-845

<table>
<thead>
<tr>
<th></th>
<th>Fallow</th>
<th>Tomato</th>
<th>Tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td>J2 per g Soil</td>
<td>94</td>
<td>64</td>
<td>7</td>
</tr>
<tr>
<td>%J2 +spores</td>
<td>47</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>Yield Kg/plot</td>
<td>Na</td>
<td>11</td>
<td>25</td>
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</table>

Two Major Problems

1) Mass production

2) Infection and host specificity
**Pasteuria penetrans** Differential Host Range Test

<table>
<thead>
<tr>
<th></th>
<th>PNG</th>
<th>PP1</th>
<th>PMJ</th>
<th>PCI</th>
<th>PP4</th>
<th>PPW</th>
<th>PPN</th>
<th>PHC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. javanica</em></td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>M. incognita</em></td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. arenaria</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>H. avenae</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td><em>H. glycines</em></td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>H. schachtii</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td><em>H. cajani</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
<td>+++</td>
</tr>
<tr>
<td><em>G. pallida</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
<td>+++</td>
</tr>
<tr>
<td><em>G. rostochiensis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

- : no attachment; +, 1-10 spores; ++, 11-20 spores; +++ > 21 spores
ENDOSPORE ATTACHMENT

Reaction of second-stage juvenile cuticle by a polyclonal antibody

*Meloidogyne incognita*  
*Meloidogyne arenaria*

Davies & Danks *Parasitology* 105, 475-480
Percentage of juveniles of *Meloidogyne arenaria* • and *M. incognita* ■ to which no spores attached

Davies *et al.*, 2001, *Parasitology* 122, 111-120
Immunofluorescence of *Pasteuria* endospores

No fluorescence  
Low fluorescence  
High fluorescence

Immunofluorescence of *Pasteururia* endospores attached to nematodes

Polyclonal antibody

Monoclonal antibody

(Adapted from Preston *et al.*, J. Nematology)
Cuticle characterisation using Mabs to *Pasteuria*

*M. incognita* Race 1: tobacco  
*M. arenaria* Race 1: peanut  

*M. javanica*: tobacco  
*M. incognita* Race 3: cotton

Cuticle characterisation using Mabs to *Pasteuria*

- *M. incognita* Race 1: tobacco
- *M. arenaria* Race 1: peanut
- *M. javanica*: tobacco
- *M. incognita* Race 3: cotton

Cuticle characterisation using Mabs to *Pasteuria*

Characterisation of endospores attached to different nematode populations by 5 Mabs

*Davies et al., Let. Appl. Microb.* 19, 370-373
Tritrophic interaction

Mixed population of nematodes: PEANUT susceptible to Race 1 of *M. arenaria*
Increase in Race 1 *M. arenaria* pathogenic *Pasteuria*

*M. arenaria* Race 1: peanut

(After Davies, K.G., 2005, *Advances in Applied Microbiology* 57, 53-78)
Tritrophic interaction

Mixed population of nematodes: TOMATO universally susceptible maintaining a diverse Pasteuria population

(After Davies, K.G., 2005, Advances in Applied Microbiology 57, 53-78)
Genomics

DNA Extraction based on robust endospores

1) Infected females dissected from roots
2) Homogenised to extract spores
3) Enzyme digestion
   Proteinase K, lysozyme, DNAase, RNAase
4) Heat treatment, proteinase K, heat treatment
5) Bead beating
6) DNA amplification using kit
Genomics

Sanger-2003  454-2008

Sequence  2.5 Mbp  8.6 Mbp
Contigs  1500  5964
Largest contig  2.5 kb  54.7 kb
GC content  62 %  45 %

Bacterial genomes range from:
< 1 Mbp Mycoplasma genitalium
to
> 9 Mbp Nostoc punctiforma
Genomics

Phylogenetic analysis undertaken using 27 full-length genes

Bacillus anthracis
Bacillus cereus
Bacillus subtilis
Bacillus halodurans
Pasteuria penetrans
Staphylococcus aureus
Lactococcus lactis
Streptococcus pyogenes
Mycoplasma pneumoniae
Mycoplasma genitalium
Mycobacterium leprae
Mycobacterium tuberculosis
Aquifex aeolicus
Campylobacter jejuni
Helicobacter pylori
Rickettsia prowazekii
Neisseria meningitidis
Xylella fastidiosa
Pseudomonas aeruginosa
Escherichia coli
Buchnera aphidicola
Pasteurella multocida
Haemophilus influenzae
Chlamyドphiola pneumoniae
Chlamyドdia trachomatis
Chlamyドdia muridarum
Borrelia burgdorferi
Treponema pallidum

Charles et al., 2005, J. Bact.
Two major problems prohibiting *Pasteuria penetrans* from being developed into a commercial product related to endospore production:

1) In vitro mass production

2) Spore 'type' and attachment specificity

"Forget about the genome. And let’s say, ‘It’s great. We’ve got all these sequences. Thank you very much for all the people that helped to get them - please get us some more.’ And let’s get on with the biology."

Sydney Brenner, January 2003, San Diego, California Plant and Animal Genome Conference
Comparison of endospore formation in *Bacillus thuringiensis* and *Pasteuria penetrans*

From Chen *et al.*, (1997) *Phytopathology* 87, 273-283
**Genomics**

- Sm all-molecule metabolism
- Broad regulatory functions
- Macromolecular metabolism
- Cell processes
- Transposons
- Other
- Conserved hypothetical
- Unknown function

6 - 7% Genomics

GO analysis

- Sporulation

Sporulation governed by Spo0F

Driks, 2002
Trends Microbiol. 10: 251
Diagram of proteins involved in the phosphorelay required for initiating the sporulation signal transduction pathway in *B. subtilis* (from Feher et al., 1997). The red arrows represent environmental signals which initiate the transfer of the phosphoryl group via the Phosphorelay pathway.
Residues in red are identical between Ppe and the Bacillus species. Residues in green are conservative exterior substitutions. The positions of b-strands and a-helices are indicated. Conserved active-site residues are highlighted in bold (arrows).
STRUCTURE OF Spo0F
charged aspartic acids line the pocket of the active site

- A divalent metal is required
- Thought to be $\text{Mg}^{2+}$ or $\text{Mn}^{2+}$
- Without the metal ion, phosphorylation will not occur

**WHAT ABOUT OTHER IONS?**

Divalent Metal Dependent Phosphorylation

Phospho-transfer from KinA~P to SpoOF

MgCl₂, Mg OF~P
ZnCl₂, Zn OFF~P
CuCl₂, Cu OF~P

Relative amount of KinA~P or SpoOF~P

Kojetin, et al., 2005, Biometals 18, 449-466
Growth of *Pasteuria* in culture is affected by divalent cations

Percentage of juveniles of *Meloidogyne arenaria* and *M. incognita* to which no spores attached

Davies *et al.*, 2001, *Parasitology* 122, 111-120
Attachment is a key determinant for infection.

*Pasteuria penetrans* endospore

*Davies, Advances in Parasitol. In prep.*

*Bacillus anthracis* endospore
### Structure of BclA collagen-like repeats and their relationship to filament length in *Bacillus anthracis*

(adapted from Sylvestre et al., *J Bact.* 185; 2003)
COMPARATIVE GENOMICS as of 2003

Gene organisation for the encoding proteins of the exosporium for *Bacillus anthracis*, *B. thuringiensis* & *B. subtilis*

Solid red genes denote BLAST hits (e\(^{-14}\)) to *Pasteuria* (dashed red e\(^{-4}\))

(Davies & Opperman, *IOBC Bulletin*, 2006, )
Gene organisation for the encoding proteins of the exosporium for Bacillus anthracis, B. thuringiensis & B. subtilis

Solid red genes denote BLAST hits ($e^{-14}$) to Pasteuria (dashed red $e^{-4}$)

(Davies & Opperman, IOBC Bulletin, 2006, )
**GENOMICS & ENDOSPORE ATTACHMENT**

>Contig1_2 (P. peniculis-147)

IFLGGTTICRLEFSGREFALGREGQOEHRRGPGTPGPPGGAHGIQGPPGPAOGIQGPGAP
GAOGIQQPPGPACTPGAQGIQQPGPAGPAGPAGPAAGSPGTGPGAPAGPAAGSPGTGPGAP
GSPGTPGPAGPACPGAPGPGTPGTGPGSPPGPAGPAGPAGPAGTPGPGAPAGPAGPAGPA
GPGAPGTPGTPGAPGPGAPGPGTPGAPGPGAPGPGAPGPGAPGPGAPGPGAPGPGAPGPG
GTQGTPQPGIQGIQPVGPGQGTGATGATGPGLNTSMTIVAGGGADTHQFITPTPEGATGAV
TLETGNGQRYGSGDQLITLDDILLSPSTGTYLMFHDNYTSAAGAVGAGAYGYSVAYF
RQFTTDFFFNOIQIVAFWGVPALVNDADFSSISNTVLGCVDIPPHGLNNHMRHESDVANS
LNTRRSQPQX

>Contig2_1 (P. peniculis-147)

LQDQTNSPFRLPDEMWSINNNHFSNHMKRKKQKIQNFHFHTFSLLGGLEDNHFMKHWIHRNGS
CIHYKNNGKIRNTITHTPSPRRSEGFRVHWIGRKSIVINSYRDQHNNHNSRTTLYR
NCEKCDNNQYEEFDNDHEFDDNHNCCDCCLNRCKCRVTGPQGTPGTPGRTGSTGRTGP
TGRTGRTGSTGRTGPTGPTGPTGPTGRTGFTGRTGSGTGSTGRTGSGTGSTGRTGGS
TGRTGPTGRTGSGTGSTGRTGSGTGSTRPLVGRRNSR

>Contig3_4 (P. peniculis-147)

VANSRLEGWTAAPAGAQGISGPPGEPIQGPACTPGAQGIQGPPGPACTPGAQGIQGPP
PAGPTGPAAGSPGTGPGAPAGPAGPAGPAAGSPGTGPGSPGTGPGAPAGPAGPAGPAGPAG
PAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAGPAG
IQPGVPQGATGATGPGLNTSMTIVAGGGADTHQFITPTPEGATGAV
TLETGNGQRYGSGDQLITLDDILLSPSTGTYLMFHDNYTSAAGAVGAGAYGYSVAYF
RQFTTDFFFNOIQIVAFWGVPALVNDADFSSISNTVLGCVDIPPHGLNNHMRHESDVANS
LNTRRSQPQX

**Total of 12 Pasteuria penetrans collagens with G-X-Y repeats**

- **79 G-X-Y repeats**
- **36 G-X-Y repeats**
- **62 G-X-Y repeats**

5 unique to RES147, 4 unique to Fl-1, 3 in common
GENOMICS & ENDOSPORE ATTACHMENT

Prediction of *Pasteuria* filament length from the equation

\[ y = 0.1829x + 3.0747 \]

(Davies & Opperman, *IOBC Bulletin*, 2006)
Spore attachment: *Velcro*-like mechanism

Davies, *Advances in Parasitology* In press.
But what about Specificity?

Spore attachment: *Velcro*-like mechanism

### Reproductive mechanisms in major groups of Root-knot nematodes *Meloidogyne* spp.

<table>
<thead>
<tr>
<th>Nematode spp.</th>
<th>Chromosome No.</th>
<th>Mode of reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. arenaria</em></td>
<td>30-46 polyploid</td>
<td>Mitotic parthenogenesis</td>
</tr>
<tr>
<td><em>M. incognita</em></td>
<td>41-46; polyploid</td>
<td>Mitotic parthenogenesis</td>
</tr>
<tr>
<td><em>M. javanica</em></td>
<td>42-48; polyploid</td>
<td>Mitotic parthenogenesis</td>
</tr>
<tr>
<td><em>M. hapla</em> Race A</td>
<td>13-17; n</td>
<td>Amphimixis &amp; meiotic parth.</td>
</tr>
<tr>
<td><em>M. hapla</em> Race B</td>
<td>43-48; polyploid</td>
<td>Mitotic parthenogenesis</td>
</tr>
</tbody>
</table>

After Evans, AAF (1998) In: The Physiology and Biochemistry of Free-living and Plant-parasitic nematodes CABI (Eds RN Perry & DJ Wright)
**Experimental design**

**M. incognita**
- Single egg mass 02-08-04
  - Routinely subcultured every 4-6 months
  - 3 spore
    - 8 single egg mass populations
      - Tested against PP3 & RES147
  - 26 spore
    - 8 single egg mass populations
      - Tested against PP3 & RES147

**M. hapla VW8 & VW9**
- Single egg mass
  - 18 generations of single egg mass
  - VW8 single juveniles
    - 2 single egg masses from 2 single J2 populations
      - Tested against PP3 & RES147
  - VW9 single juveniles
    - 2 single egg masses from 3 single J2 populations
      - Tested against PP3 & RES147

*Davies, Rowe & Williamson 2008 Int. J. Parasit., 38, 851 - 859*
Endospore attachment of *Pasteuria* populations RES147 & PP3 to *Meloidogyne hapla* strains VW8 & VW9 and *M. incognita*.

ANOVA $P < 0.001$

Davies, Rowe & Williamson 2008 *Int. J. Parasit.*, 38, 851 - 859
Spores, RES147 & PP3, attaching to single juvenile descent lines arising of *M. hapla* VW9K1 (light grey), VW9K2 (dark grey), VW9K3 (black)

Segregation in spore attachment

ANOVA $P < 0.001$

Davies, Rowe & Williamson 2008 *Int. J. Parasit.*, 38, 851 - 859
Endospore attachment of *Pasteuria* population PP3 to single juvenile descent lines of *Meloidogyne incognita*

Somaclonal variation in spore attachment

ANOVA $P < 0.001$

Davies, Rowe & Williamson 2008 *Int. J. Parasit.*, 38, 851 - 859
Endospore attachment of *Pasteuria* population RES147 to *Meloidogyne incognita*

Somaclonal variation in spore attachment

ANOVA $P < 0.001$

Davies, Rowe & Williamson 2008 *Int. J. Parasit.*, 38, 851 - 859
Relationship between juvenile age and spore attachment

Davies et al., 1991 Revue de Nematol 14, 616 - 618.
DAF-2, IGF & innate immunity in worms

Antimicrobial defences in C. elegans. (a) Basic anatomy of C. elegans. Of particular note are the physical barriers, the grinder that mechanically disrupts the bacteria that form a worm's normal diet, and the cuticle that envelopes the animal, both of which protect the worm from microbial aggression, and the pseudocoelom, a fluid-filled cavity that separates the intestinal cells from the hypodermis. (b) A model for the cellular basis of innate immunity in C. elegans. The presence of pathogens in the environment is perceived via the sensory neurons, which generate a signal that is transmitted to target tissues via the pseudocoelom. Supporting such an idea is the fact that, in contrast to their ligands, which are secreted factors expressed in the nervous system, the different proteins involved in the DAF-2 and DBL-1 signalling cascades (see Figure 1) are expressed in the intestine and hypodermis, as are putative antimicrobial proteins, such as LYS-8 and R09B5.3. It is possible that the establishment of an infection in the intestinal lumen also plays a role in triggering a defence reaction, via an as yet uncharacterised mechanism. It is hypothesised that antimicrobial proteins and peptides are secreted into the intestine, via specialised vesicular traffic, as illustrated for LYS-8.

Anne Millet and Jonathan Ewbank 2004 Curr. Opin. Immunol. 16, 4-9
An evolutionary conserved phosphorylation cascade involving the insulin/insulin-like growth factor (IGF) receptor is well characterised in *C. elegans*.

Activation of phosphorylation pathway begins with binding of insulin-like ligand to DAF-2 receptor (38 present in Ce only a few characterised)

DAF-2 activates AGE-1 which converts phosphatidylinositol biphosphate to a triphosphate

PiP$_3$ binds to the AKT-1/AKT2 complex that phosphorylates the Forkhead transcription factor DAF-16

DAF-16+P cannot be translocated to nucleus to activate DAF-2 pathways

DAF-16 can enter nucleus
L1 and L2 activates dauer formation
L3 adults activates stress response and anti-microbial genes

(Millet & Ewbank, 2004)
DAF-2 mutants can alter a number of important metabolic activities:

- Alter lipid metabolism
- Alter fertility
- Alter lifespan
- Dauer formation
- Activate stress response

Inhibition of DAF-2
DAF-2 mutants can alter a number of important metabolic activities.

- Inhibition of DAF-2
- Alter lifespan
- Alter lipid metabolism
- Dauer formation
- Alter fertility
- Activate stress response
EPL001 is a peptide that inhibits IGF:

72 hours after application, EPL001 inhibits Epidermal Growth Factor (EGF) & Insulin Growth Factor (IGF) stimulated MCF-7 cells (breast carcinoma cell line).

John Haylor et al., in prep.
Manipulation of life-span and fecundity in *C. elegans*

**Mean larvae per adult**

Control  EPL001  EPL030  
17  24  6  
+43%  -64%

ANOVA  $P < 0.05$

Davies and Hart (2008) *Nematology* 10, 103-112
The effects of EPL001 and EPL030 on the attachment of *Pasteuria* to root-knot J2s

- *Meloidogyne incognita* allowed to hatch in water
- Treat with 1µM of EPL001 and EPL030 (water control)
- At 0, 18, 21 and 27 hrs wash (x3) water
- Endospore (strain RES147) attachment test by centrifugation
- Count endospores adhering to the cuticle
The effects of EPL001 and EPL030 on the attachment of *Pasteuria* to root-knot J2s

**ANOVA \( P < 0.001 \)**

- **T0**
- **T18**
- **T21**
- **T27**

Davies unpublished
Modulation of surface coat through EPL001 and EPL030

Excreteory/secretory products

DAF-2 modulation of E/S products

Davies unpublished
Three types of cuticle variation

• **Constitutive variation:** under genetic control and can segregate

• **Clonal variation:** probable under epigenetic control

• **Induced variation:** modulated by environmental parameters
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