The Dynamic Nematode Cuticle: what can *Pasteuria* endospores teach us?

Keith G Davies

AAB Advances in Nematology

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http://www.go.herts.ac.uk/keithdavies
http://www.kgdavies.com
Plant-parasitic nematodes

*Pasteuria penetrans* is potentially a control agent
Cuticle structure as extracellular matrix

Adapted from: Kramer JM (1997)  
*C. elegans Book II* Chapter 17

Highly structured extracellular matrix
Nematode surfaces are targets for environmental assault: Abiotic & Biotic

Nematode has a large number of different collagens
Labile surface coat

Surface Coat printing

The surface coat appears dynamic in that it is synthesised continuously and sloughed off.

Curtis et al 2011 Plant Nematode Surfaces In Biological Control of Plant-parasitic Nematodes (Davies K & Spiegel Y Eds) Springer
Bacillus cereus family endospore (TEM)

Courtesy Anne Moir
A schematic diagram illustrating a possible model for the exosporium of the *B. cereus* family.
Endopore coat structure

Adapted from Colderon-Romero et al., (2018) Clostridium difficile exosporium... https://doi.org/10.1371/journal.ppat.1007199
## GENOMICS & ENDOSPORE ATTACHMENT

### Bcl-A

<table>
<thead>
<tr>
<th>B. anthracis strain</th>
<th>G-x-y repeat region</th>
<th>Number of G-x-y repeats</th>
<th>Filament length (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>770</td>
<td>314</td>
<td>445</td>
<td>162</td>
</tr>
<tr>
<td>818</td>
<td>260</td>
<td>391</td>
<td>130</td>
</tr>
<tr>
<td>Ames</td>
<td>251</td>
<td>382</td>
<td>124</td>
</tr>
<tr>
<td>53169</td>
<td>239</td>
<td>370</td>
<td>118</td>
</tr>
<tr>
<td>9602R, A2R, 6183R</td>
<td>131</td>
<td>262</td>
<td>52</td>
</tr>
<tr>
<td>6602</td>
<td>122</td>
<td>253</td>
<td>48</td>
</tr>
<tr>
<td>5725R</td>
<td>113</td>
<td>244</td>
<td>42</td>
</tr>
<tr>
<td>4229</td>
<td>92</td>
<td>223</td>
<td>28</td>
</tr>
</tbody>
</table>

Adopted from Sylvestre, P. et al., 2003
Spore attachment: *Velcro*-like mechanism

Davies, 2009, *Advances in Parasitology* 68, 211-245
Three PhD students

Arohi Srivastava

Victor Phani

Jamie Orr

*Pasteuria* attachment

*Pasteuria* transcriptome

*Pasteuria* (meta-)genome

Two sides of a host parasite interaction
MICROBIAL GENOMICS

Research paper template

De novo assembly of the Pasteuria penetrans genome reveals high plasticity, host dependency, and BclA-like collagens.

Jamie N Orr¹,², Tim H Mauchline³, Peter J Cock¹, Vivian C Blok¹, and Keith G Davies²,⁴

¹Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK.; ²School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK.; ³Sustainable Agriculture Sciences, Rothamsted Research, Harpenden, AL5 2JQ, UK.; ⁴Norwegian Institute of Bioeconomy Research, Postboks 115 NO-1431, As, Norway.

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Keywords: Pasteuria, PacBio, MDA, Biocontrol, Meloidogyne.

1. ABSTRACT

Pasteuria penetrans is a gram-positive endospore forming bacterial parasite of Meloidogyne.
### Fewer endospore related genes present in ‘non pathogenic’ *Bacillus* spp.

<table>
<thead>
<tr>
<th>Selected Genes</th>
<th><em>B. cereus</em> (13)</th>
<th><em>B. thuringiensis</em> (12)</th>
<th><em>B. anthracis</em> (7)</th>
<th><em>B. subtilis</em> (12)</th>
<th><em>B. licheniformis</em> (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rfbA</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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<tr>
<td>rfbB</td>
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<td>100%</td>
<td>86%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>yjcC</td>
<td>100%</td>
<td>100%</td>
<td>86%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>bclA</td>
<td>100%</td>
<td>100%</td>
<td>86%</td>
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<td>50%</td>
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<tr>
<td>rfbD</td>
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<td>100%</td>
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<tr>
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<tr>
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<td>100%</td>
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<tr>
<td>exsE</td>
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<tr>
<td>cloP</td>
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<td>0%</td>
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<tr>
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<td>92%</td>
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</tr>
<tr>
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<td>92%</td>
<td>86%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
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<td>92%</td>
<td>92%</td>
<td>86%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>exsB</td>
<td>92%</td>
<td>83%</td>
<td>86%</td>
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</tr>
<tr>
<td>exsG</td>
<td>92%</td>
<td>75%</td>
<td>71%</td>
<td>8%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Fig. 2:** Percentage occurrence of 15 selected endospore-related genes across 46 different strains of five species of *Bacillus* spp. Red highlighted genes (*bclA* and *exsJ*) denote collagen-like sequences.
Table 1. List of 17 putative CLPs identified in Pasteuria penetrans RES 148

<table>
<thead>
<tr>
<th>Putative collagen</th>
<th>Length</th>
<th>N-terminal</th>
<th>C-terminal</th>
<th>Number of GXY repeat regions and their location within the region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppc1</td>
<td>1215 bp/405 aa</td>
<td>MSNLELLHRLCC</td>
<td>RQVVIELPSGN</td>
<td>83 CVCPP (7..11) CVCPP (39..43)</td>
</tr>
<tr>
<td>Ppc8</td>
<td>753 bp/251 aa</td>
<td>MPNHSGLRGSPL</td>
<td>GFVGVLVENRGGL</td>
<td>30 SPV (4..6)</td>
</tr>
<tr>
<td>Ppc9</td>
<td>669 bp/223 aa</td>
<td>MISVVVTMTSPL</td>
<td>SRSPHAEMDYLP</td>
<td>14 TPVTPVIPVIPVIPV (7..24) DPVAP (28..32) V (36) NPVPNPV (46..51) DPV (64..66) NPV (73..75)</td>
</tr>
<tr>
<td>Ppc16</td>
<td>606 bp/202 aa</td>
<td>MYHNDYQGKMSD</td>
<td>PCPPPYPHYREY</td>
<td>28 None</td>
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<tr>
<td>Ppc17</td>
<td>1242 bp/414 aa</td>
<td>MKRSTKYPFLAM</td>
<td>GQAANLIIRRVF</td>
<td>20 ST (25..26)</td>
</tr>
<tr>
<td>Ppc18</td>
<td>1170 bp/390 aa</td>
<td>MKIKTLMLLIG</td>
<td>TTSISMYVRQIA</td>
<td>23 None</td>
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<tr>
<td>Ppc19</td>
<td>1143 bp/381 aa</td>
<td>MIMKAILNIYLI</td>
<td>TAASSLIKRIAS</td>
<td>27 NLQ (73..76)</td>
</tr>
<tr>
<td>Ppc20</td>
<td>780 bp/260 aa</td>
<td>MRGNARIGINLI</td>
<td>RATASVIRQIF</td>
<td>28 None</td>
</tr>
<tr>
<td>Ppc21</td>
<td>1938 bp/646 aa</td>
<td>MLEFHLPESSYI</td>
<td>SSGASFTIRRVA</td>
<td>129 IT (19..20)</td>
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<tr>
<td>Ppc23</td>
<td>1158 bp/386 aa</td>
<td>MLAVLSSLPLCA</td>
<td>SISASVLVIRRA</td>
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<tr>
<td>Ppc24</td>
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<td>MNEVTQLSQADY</td>
<td>GTAFLSMIRRLN</td>
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<tr>
<td>Ppc25</td>
<td>1179 bp/393 aa</td>
<td>MKKIIYLLIS</td>
<td>SINASILIQIS</td>
<td>15 None</td>
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<tr>
<td>Ppc26</td>
<td>837 bp/279 aa</td>
<td>MASLNKVRVQQL</td>
<td>TATQANLFFKL</td>
<td>15 None</td>
</tr>
<tr>
<td>Ppc28</td>
<td>843 bp/281 aa</td>
<td>MILNLFPCCGF</td>
<td>VTITKYSIDSICS</td>
<td>26 None</td>
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<tr>
<td>Ppc29</td>
<td>837 bp/279 aa</td>
<td>MILNLFPCCGF</td>
<td>VFQYSTNICISQ</td>
<td>29 TFT (79..81)</td>
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<tr>
<td>Ppc30</td>
<td>774 bp/258 aa</td>
<td>MLIGGNNLFGNT</td>
<td>GTAFLSITIRLN</td>
<td>30 I (3) IT (14..15)</td>
</tr>
<tr>
<td>Ppc33</td>
<td>1770 bp/590 aa</td>
<td>MSRSQNNIINY</td>
<td>SQKTWILIEQIY</td>
<td>49 AEK (124..126)</td>
</tr>
</tbody>
</table>

Pre-incubation of *Pasteuria* endospores

Arohi Srivastata 2017 *PhD Thesis, University of Hertfordshire*
A transcriptomic snapshot of early molecular communication between Pasteuria penetrans and Meloidogyne incognita

Victor Phani, Vishal S. Somwanshi, Rohit N. Shukla, Keith G. Davies, and Uma Rao

Background: Southern root-knot nematode Meloidogyne incognita (Kofoid and White, 1919), Chitwood, 1949 is a key pest of agricultural crops. Pasteuria penetrans is a hyperparasitic bacterium capable of suppressing the nematode reproduction, and represents a typical coevolved pathogen-parasitoid system. Attachment of Pasteuria endospores to the cuticle of second-stage nematode juveniles is the first and pivotal step in the bacterial infection. RNA-Seq was used to understand the early transcriptional response of the root-knot nematode at 8 h post Pasteuria endospore attachment.

Results: A total of 52,485 transcripts were assembled from the high quality (HQ) reads, out of which 582 transcripts were found differentially expressed in the Pasteuria endospores. The expression profiles of 582 transcripts were validated by qRT-PCR. RNAi based silencing of transcripts coding for fructose bi-phosphate aldolase and glucosyl transferase resulted in higher incidence of endospore attachment as compared to the controls, whereas, silencing of aspartic protease and ubiquitin coding transcripts resulted in higher incidence of endospore attachment on the nematode cuticle.
Knockdown of a mucin-like gene in *Meloidogyne incognita* (Nematoda) decreases attachment of endospores of *Pasteuria penetrans* to the infective juveniles and reduces nematode fecundity.

Victor Phani, Tagginahalli N. Shivakumara, Keith G Davies, Uma Rao


This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mpp.12704

**Sugar inhibition assay**

**Knockdown mucin decreases endospore attachment**

Phani *et al.*, (2018) *Molecular Plant Pathology*
Meloidogyne incognita Fatty Acid- and Retinol- Binding Protein (Mi-FAR-1) Affects Nematode Infection of Plant Roots and the Attachment of Pasteuria penetrans Endospores

Victor Phani¹, Tagginahalli N. Shivakumara¹, Keith G. Davies²,³* and Uma Rao¹*

¹Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, India
²School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom
³Norwegian Institute of Bioeconomy Research, Ås, Norway

Feeding dsRNA Mj-FAR-1 increased Pasteuria attachment

Phani et al., 2017 Frontiers in Microbiology
doi.org/10.3389/fmicb.2017.02122
Spore attachment more complicated than a simple one to one (protein to protein??)  
*Velcro*-like mechanism

Davies, 2009, *Advances in Parasitology* 68, 211-245
Trap crops:

Examples

Control of stem borers and in maize using push-pull

This is a well developed system that exemplifies how cultural methods integrated with several other approaches can be used to control pests and weeds

Trap cropping:
Control of stem borer using “Push-pull”

Crop habitat diversification

Main crop = Maize/sorghum

Intercrop = *Desmodium* spp (Silver or Green leaf) or Molasses Grass (*Melinis minutiflora*)

Trap crop = Napier grass (*Pennisetum purpureum*)

Repellent none host intercrop = *Desmodium* spp or Molasses grass

Attractant host trap crop = Napier grass or Sudan grass (also attractant to stem borer parasitic wasps (*Cotesia* spp))

Can plant root exudates change the nematode cuticle to become more susceptible and enhance hyperparasitism?

**Nematodes**
- Polyphagous
- Host specific

**Pasteuria**
- Highly specific

**Root exudates**
- Preferred-host
- Moderate-host
- Non-host

Specific response

Generalized / specific response

Not clear?
Can a susceptible host recruit *Pasteuria* to reduce nematode populations compared to a non-host?

**Susceptible Host**
- Tomato

**Poor/Non Host**
- Potato

**Pasteuria**

- J2 threat to plants
- J2 not a threat
# Experimental system

## Plant Nematode

<table>
<thead>
<tr>
<th>Plant Nematode</th>
<th>Cowpea</th>
<th>Tomato</th>
<th>Potato</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. incognita</em> Race 1</td>
<td>Partial host</td>
<td>host</td>
<td>Poor host</td>
</tr>
<tr>
<td><em>Pasteuria penetrans</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ex. RKN</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. cajani</em></td>
<td>Host</td>
<td>Poor host</td>
<td>Poor host</td>
</tr>
<tr>
<td><em>Pasteuria nishizawae</em>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ex. H. cajani</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Methods
Characterization of root exudates of cowpea, tomato and potato plants by Gas Chromatography/ Mass spectrometry (GC/MS)
Standard *Pasteuria* spore attachment on *M. incognita* Race 1 J2

As the J2 ages and starves the nematode surface coat dynamics altered to reduces *Pasteuria* attachment.
As the J2 aged and starved, host root exudates stimulated the surface coat to enhance attachment.
Exposing *H. cajani* J2 to root exudates

- **Cowpea**: Fresh J2, 7 days old J2, 14 days old J2
- **Tomato**: Fresh J2, 7 days old J2, 14 days old J2
- **Potato**: Fresh J2, 7 days old J2, 14 days old J2

- **Susceptible host**
  - Cowpea: 24.4
  - Tomato: 73.8
  - Potato: 90.6

- **Non host**
  - Cowpea: -6.52
  - Tomato: -57.6
  - Potato: -77.5

- **Non host**
  - Cowpea: 22.8
  - Tomato: -65.14
  - Potato: -4.34

- **Potato exudates significantly influenced HCP attachment**
- **HCP is a common parasite of *H. cajani* and *Globodera spp***
- **Non host tomato exudates significantly reduced attachment**
Characterisation of compounds by GC/MS

Tomato
  cv S22

Cowpea
  cv Pusa Komal

Potato
  cv Chandramukhi

38
13
8
14
3
13
40
55
23
10
Nematode cuticle is highly dynamic and responds to environmental stimuli such as plant exudates.

Some evidence that host plants can recruit a hyperparasite.

It appears to do this by decreasing the rate at which cuticles age.

Knockdown of various cuticle proteins affect endospore attachment both positively and negatively.

Endospore adhesion is complicated; many more questions to answer.

What can *Pasteuria* endospore adhesion teach us?