Biological Control and *Pasteuria penetrans*:

genomics and molecular biology

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ROTHAMSTED
RESEARCH

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Structure of talk:

•Introduction and context

•Immunological approach to host specificity

•Genomics and taxonomy

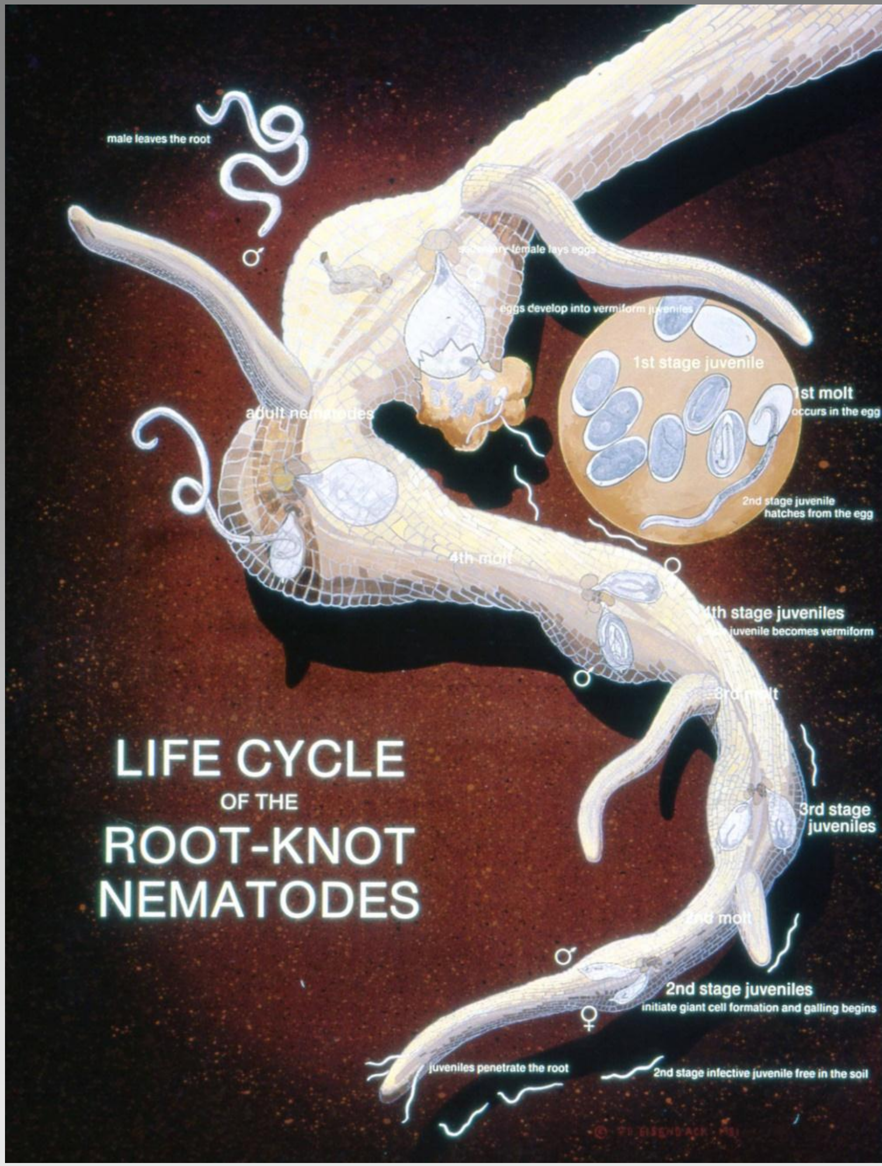
•*In vitro* culture

•Collagen and the mechanism of attachment

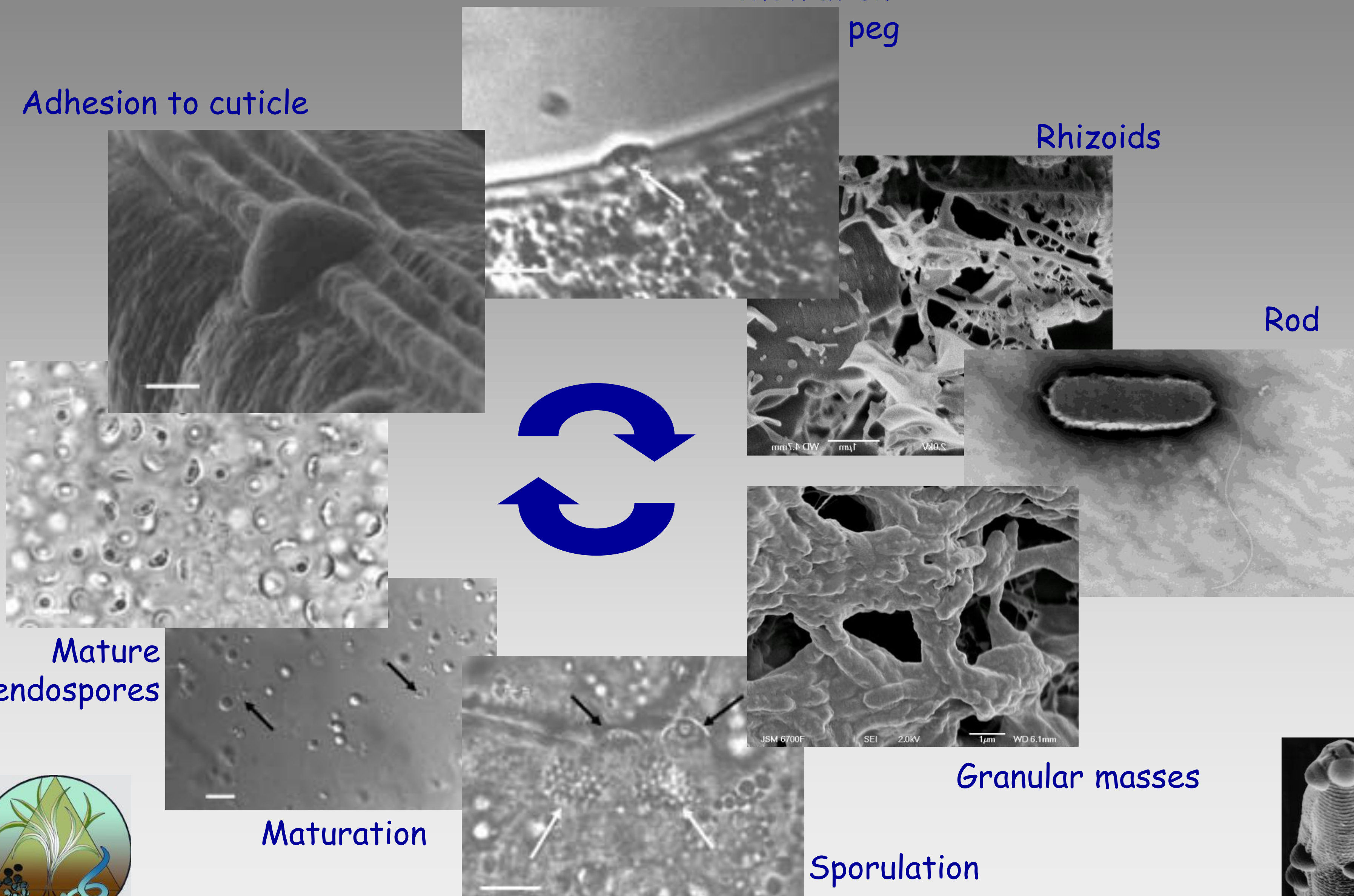
•Three types of cuticle variation

ROTHAMSTED RESEARCH

Generalised life cycle of a root-knot nematode *Meloidogyne* spp.



The life-cycle of *Pasteuria penetrans* on root-knot nematodes (*Advances in Parasitol. In press*)



Increase in tomato yield following application of *Pasteuria penetrans* to root-knot infected soils in Equador

adapted from Trudgill *et al.*, (2000) *Nematology* 2, 823-845

	Fallow	Tomato	Tomato
J2 per g Soil	94	64	7
%J2 +spores	47	89	100
Yield Kg/plot	Na	11	25

Two Major Problems

1) Mass production

2) Infection and host specificity

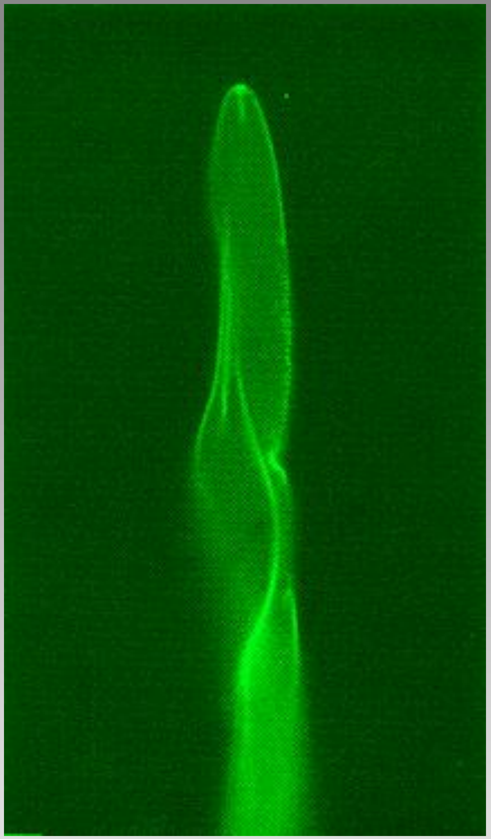
Pasteuria penetrans Differential Host Range Test

	PNG	PP1	PMJ	PCl	PP4	PPW	PPN	PHC
M. javanica	+++	++	++	++	+	+	+	-
M. incognita	+++	++	++	++	+	-	-	-
M. arenaria	+	+	+	+	+	-	-	-
H. avenae	-	-	-	-	+	+	-	NA
H. glycines	-	-	NA	-	-	+	++	+
H. schachtii	-	-	-	-	-	+	+	++
H. cajani	-	-	-	-	-	NA	NA	+++
	-	-	-	-	-	NA	NA	+++
G. pallida								
G. rostochiensis	-	-	-	-	-	++	+	+++

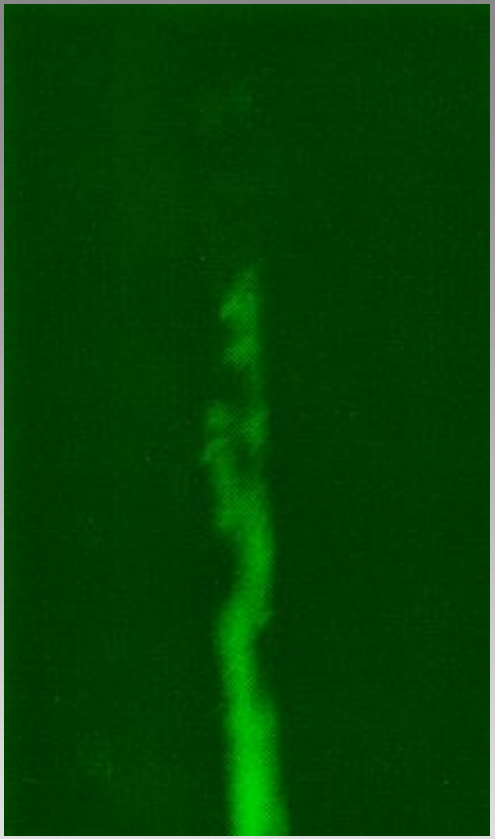
-, no attachment; +, 1-10 spores; ++, 11-20 spores; +++ > 21 spores

ENDOSPORE ATTACHMENT

Reaction of second-stage juvenile cuticle by a polyclonal antibody



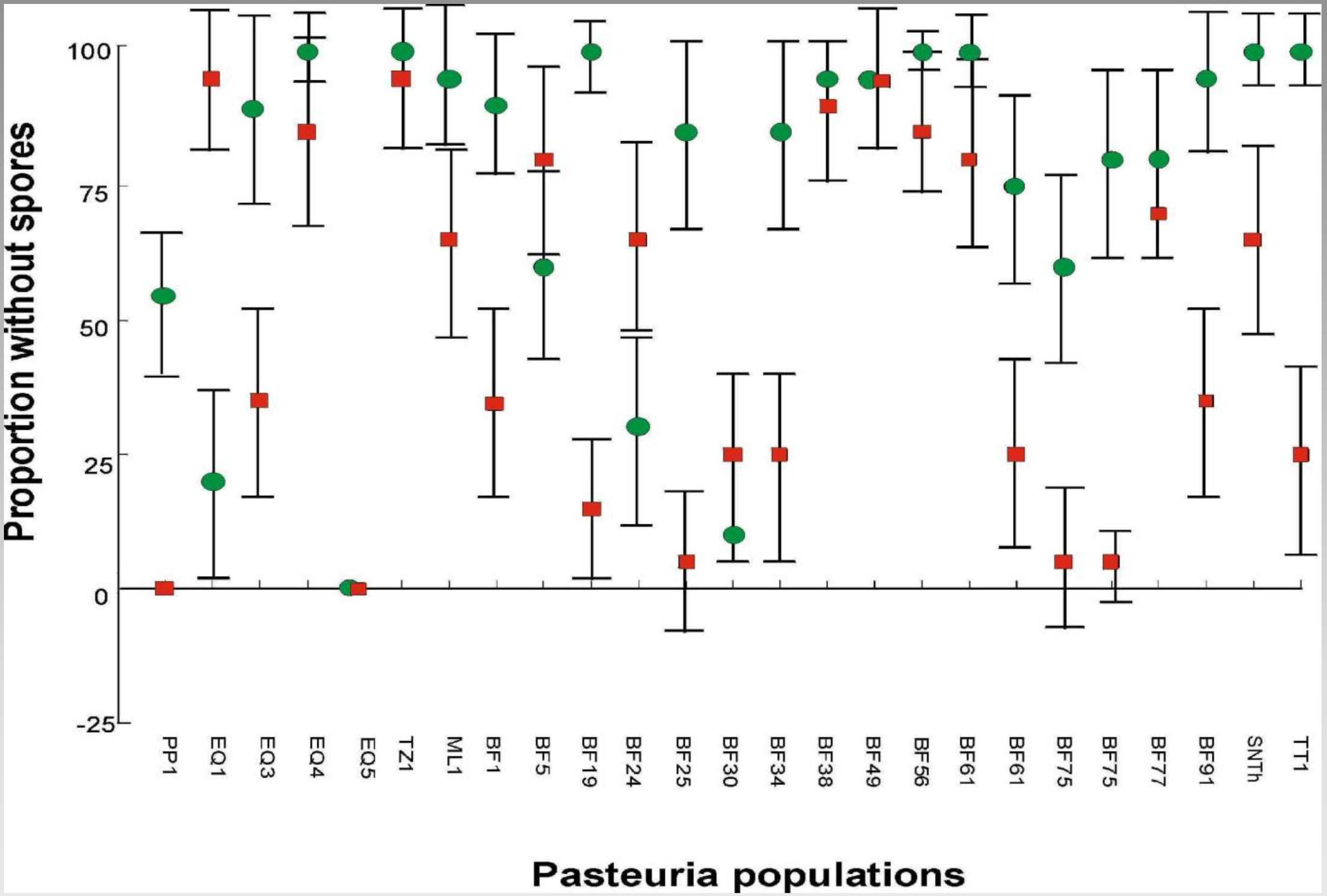
Meloidogyne incognita



Meloidogyne arenaria

Davies & Danks *Parasitology* 105, 475-480

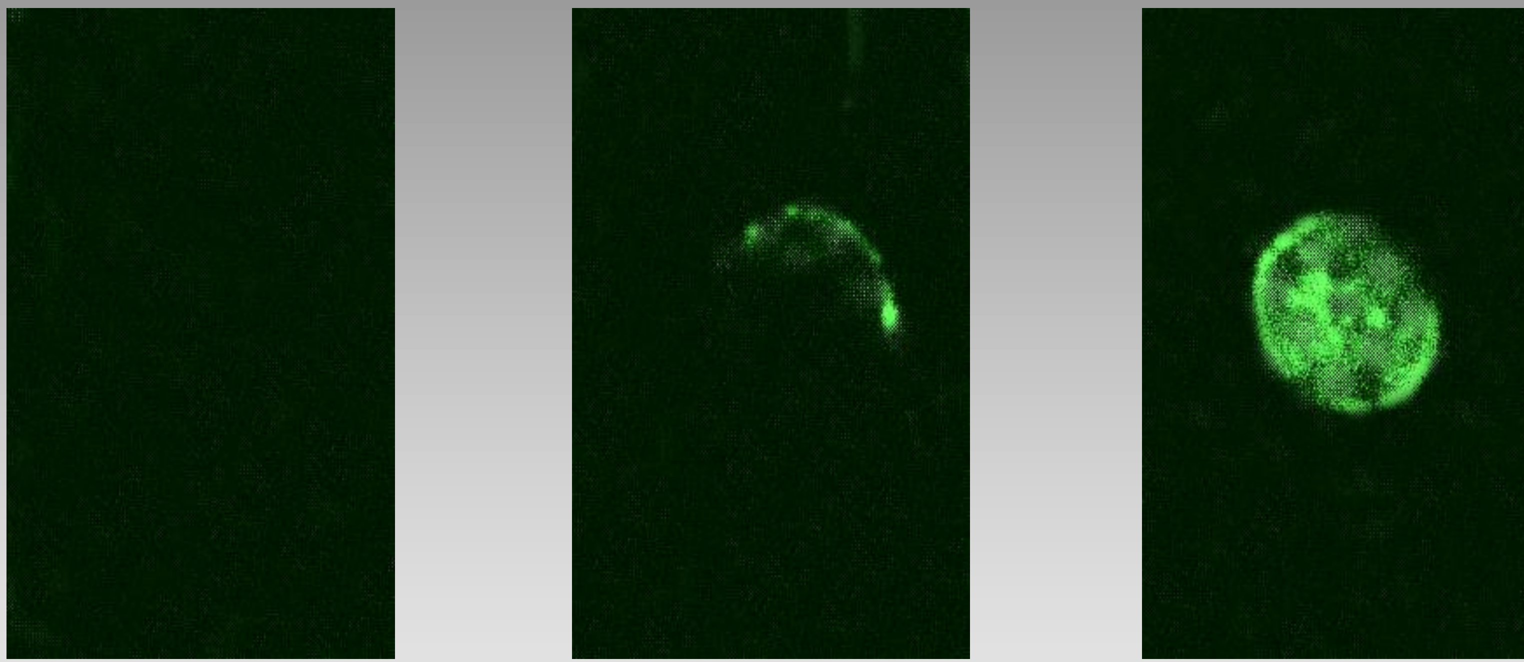
Percentage of juveniles of *Meloidogyne arenaria* and *M. incognita* to which no spores attached



Davies *et al.*, 2001, *Parasitology* 122, 111-120

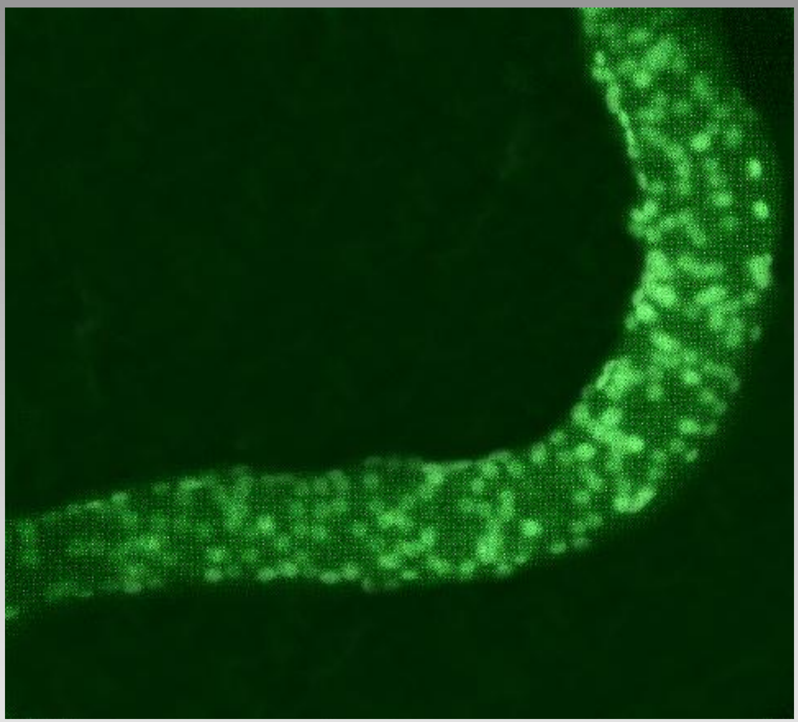
Immunofluorescence of *Pasteuria* endospores

No fluorescence | Low fluorescence | High fluorescence

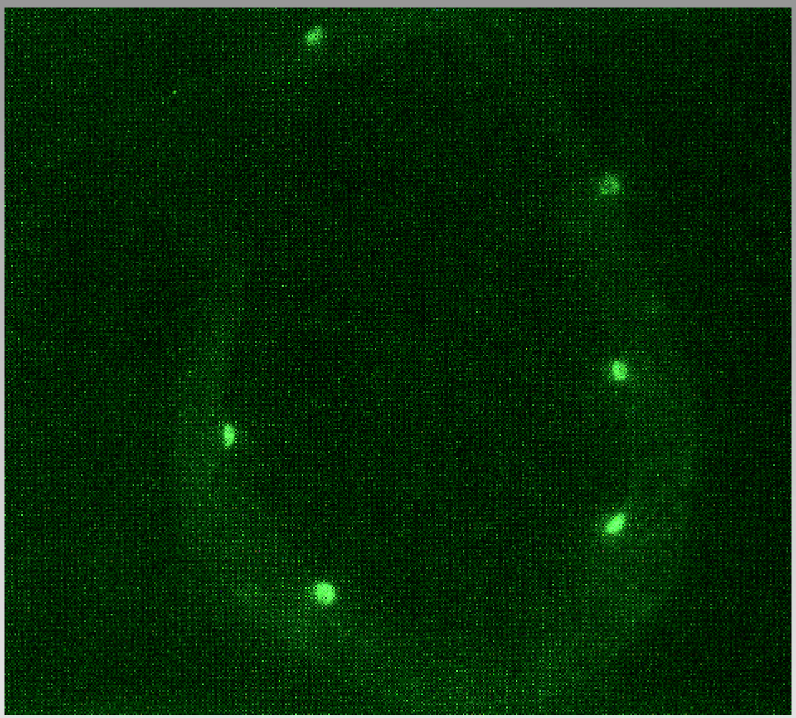


Davies *et al.*, *Let. Appl. Microb.* 19, 370-373

Immunofluorescence of *Pasteuria* endospores attached to nematodes



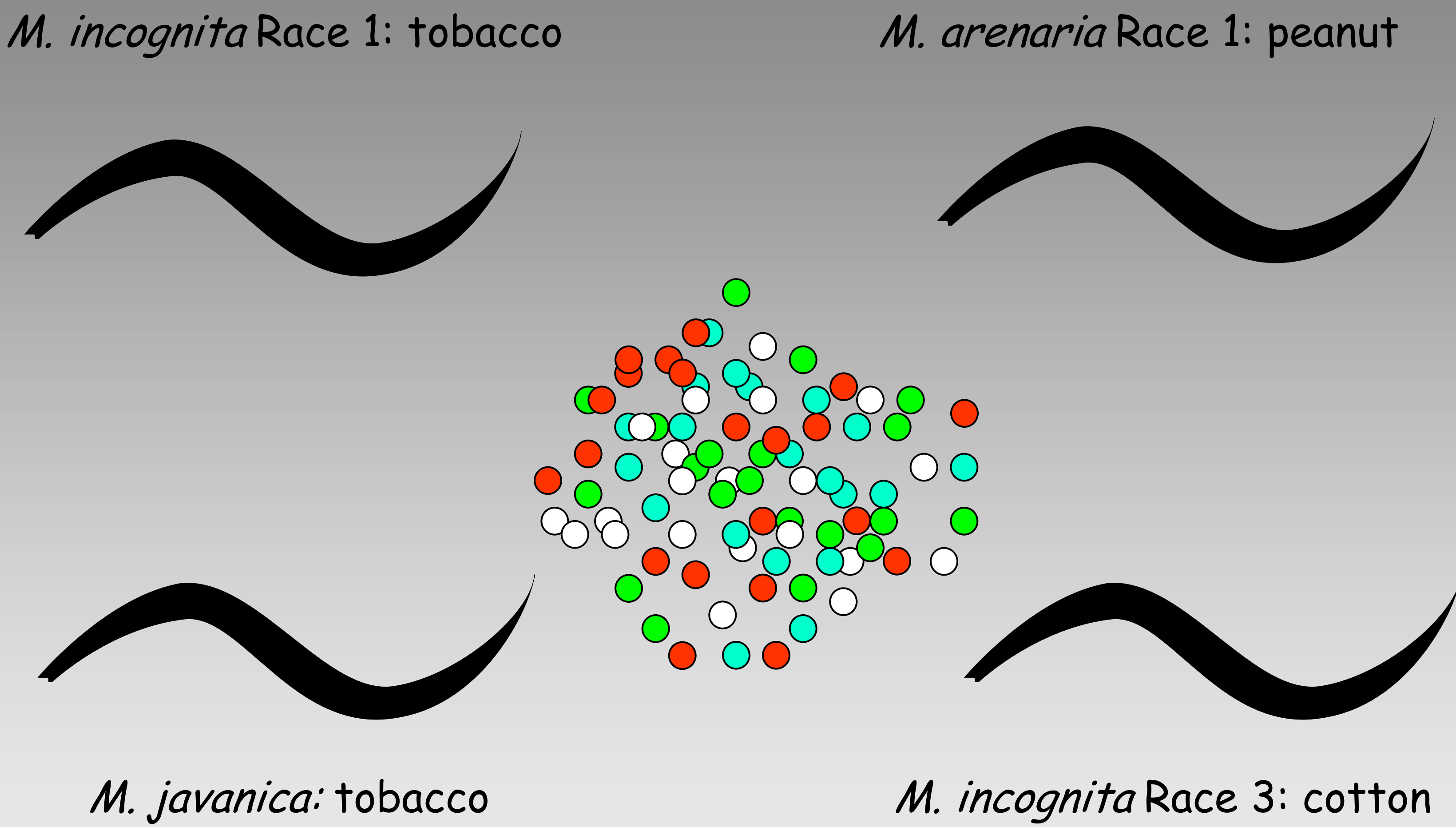
Polyclonal antibody



Monoclonal antibody

(Adapted from Preston *et al.*, J. Nematology)

Cuticle characterisation using Mabs to *Pasteuria*

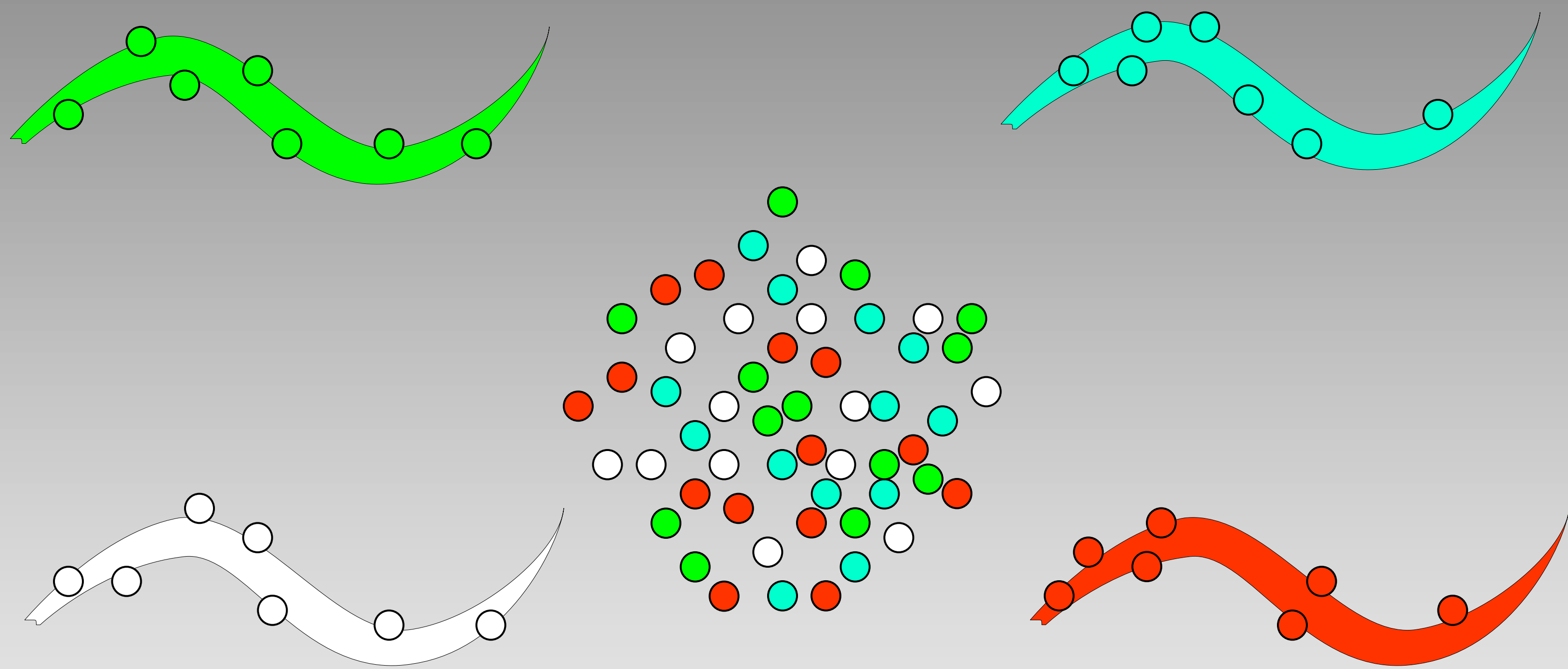


Davies *et al.*, *Let. Appl. Microb.* 19, 370-373

Cuticle characterisation using Mabs to *Pasteuria*

M. incognita Race 1: tobacco

M. arenaria Race 1: peanut

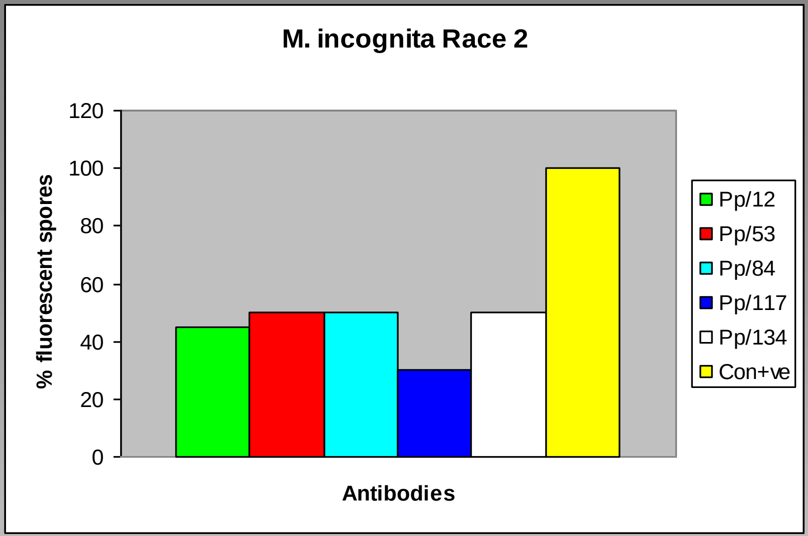


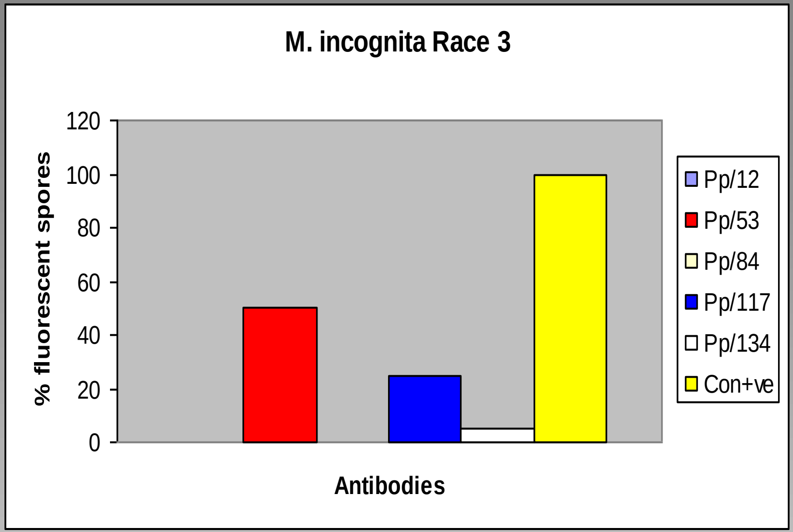
M. javanica: tobacco

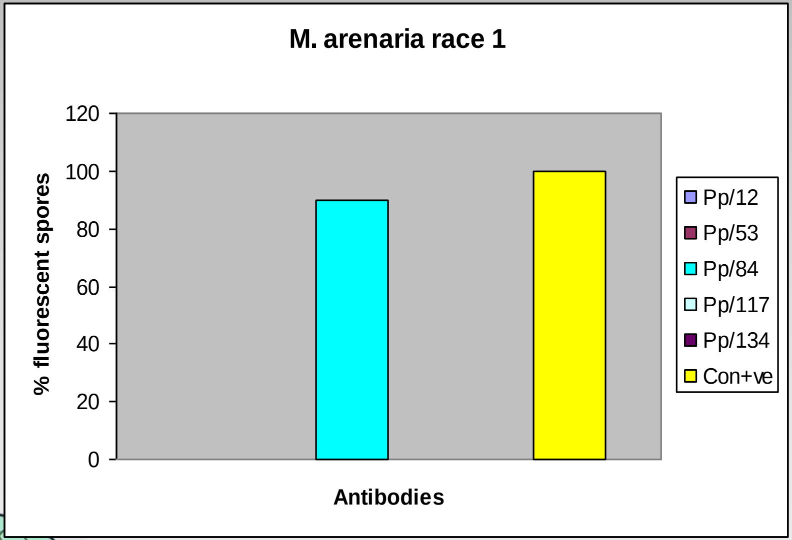
M. incognita Race 3: cotton

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

Cuticle characterisation using Mabs to *Pasteuria*





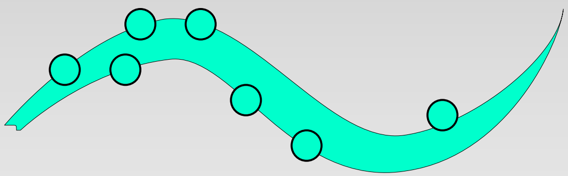


Characterisation of endospores attached to different nematode populations by 5 Mabs

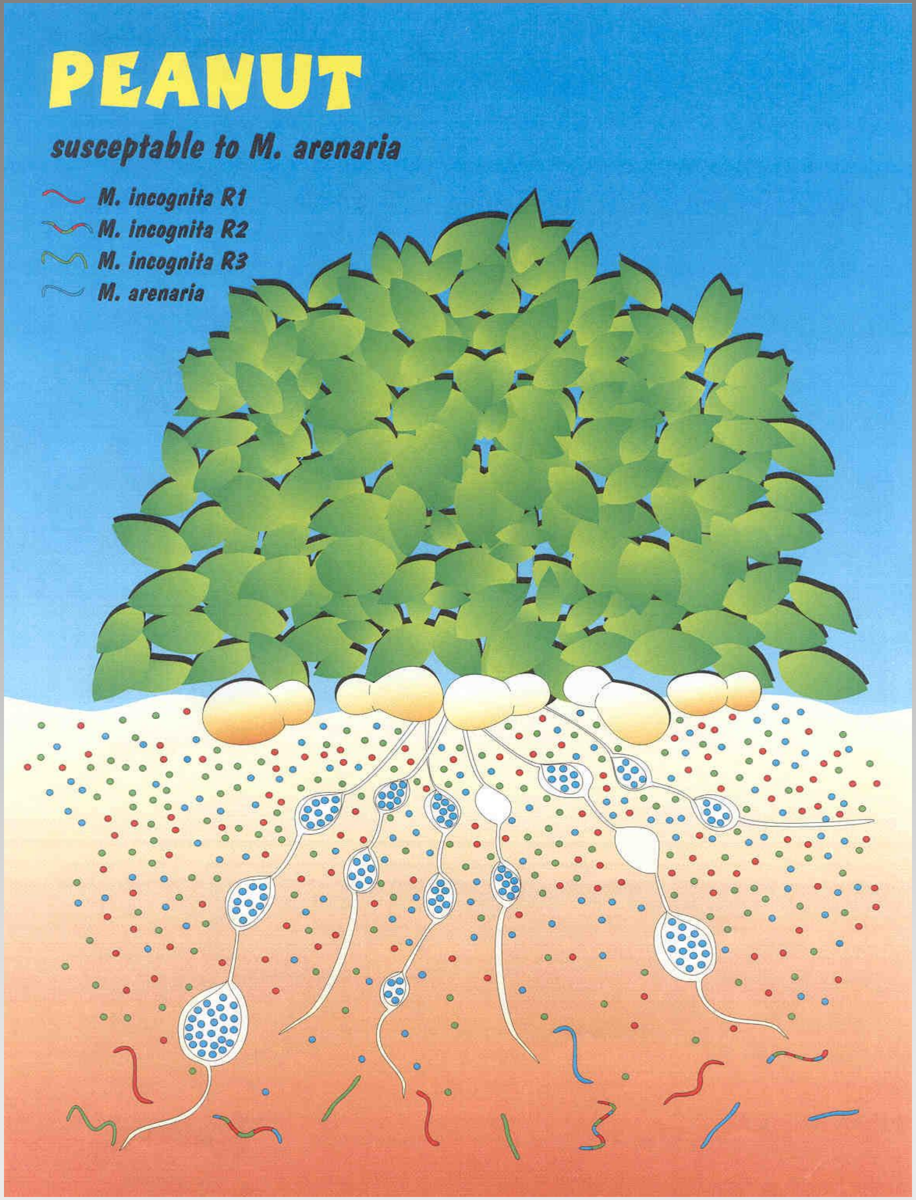
Davies *et al.*, *Let. Appl. Microb.* 19, 370-373

Tritrophic interaction

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



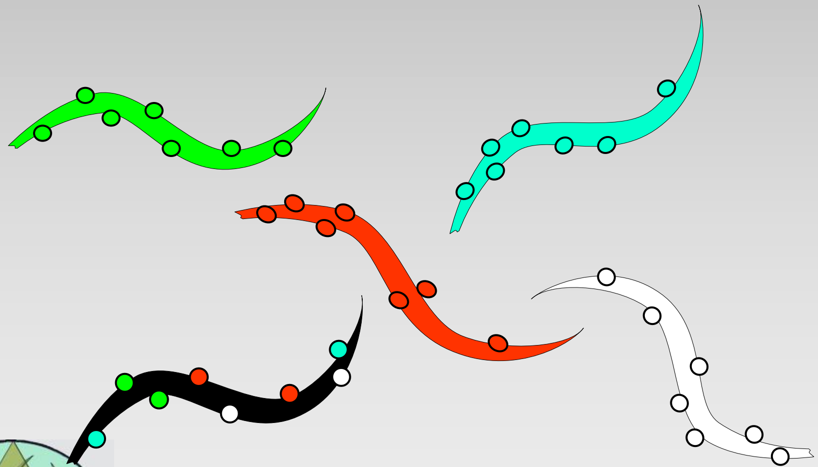
M. arenaria Race 1: peanut

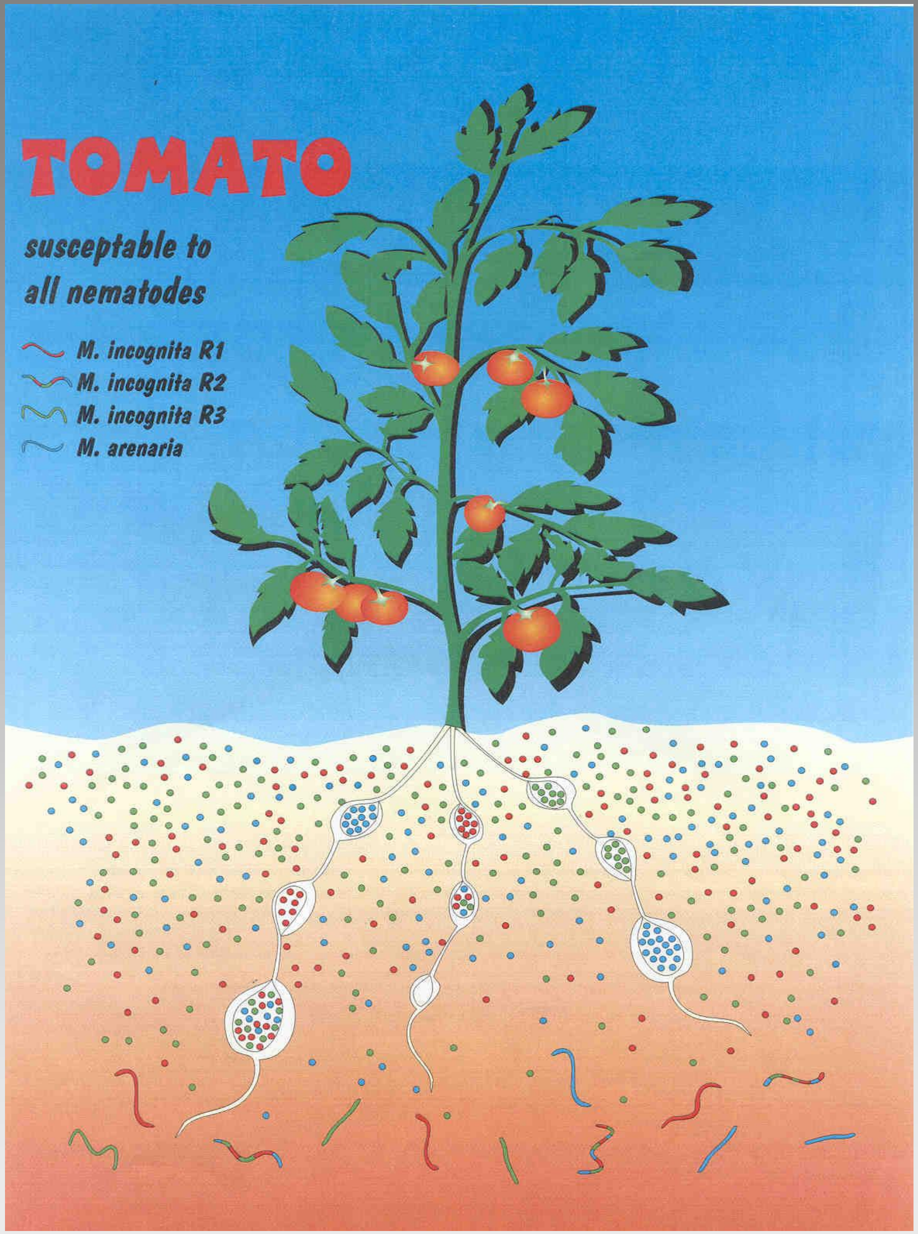


(After Davies, K.G., 2005, *Advances in Applied Microbiology* 57, 53-78)

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 by
bead beating

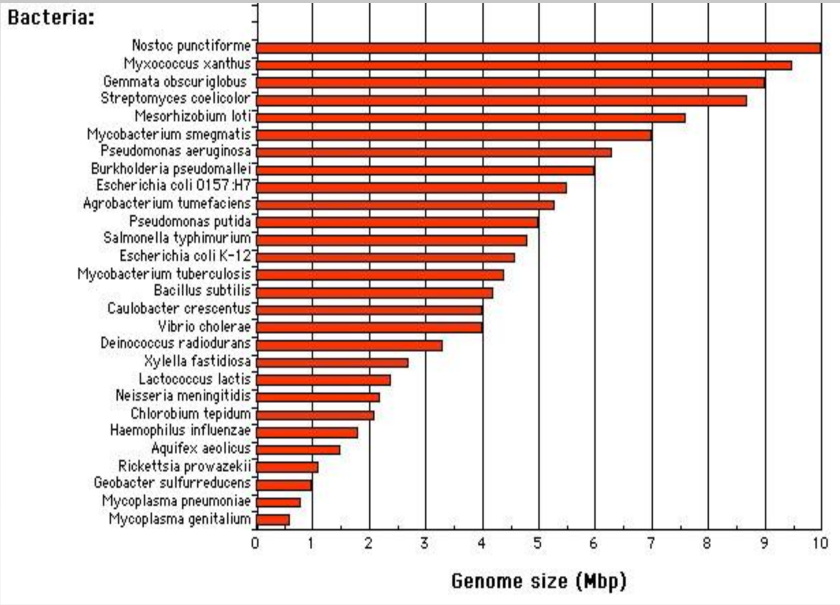
DNA Extraction based on robust endospores

1) Infected females dissected from roots
2) Homogenised to extract spores
3) Enzyme digestion
   Proteinase K, lyzozyme, DNAase, RNAase
4) Heat treatment, protinase K, heat treatment
5) Bead beating
6) DNA amplification using kit
7) Sequencing: Sanger (2003) and 454 (2008)

Genomics

THE BIG PICTURE

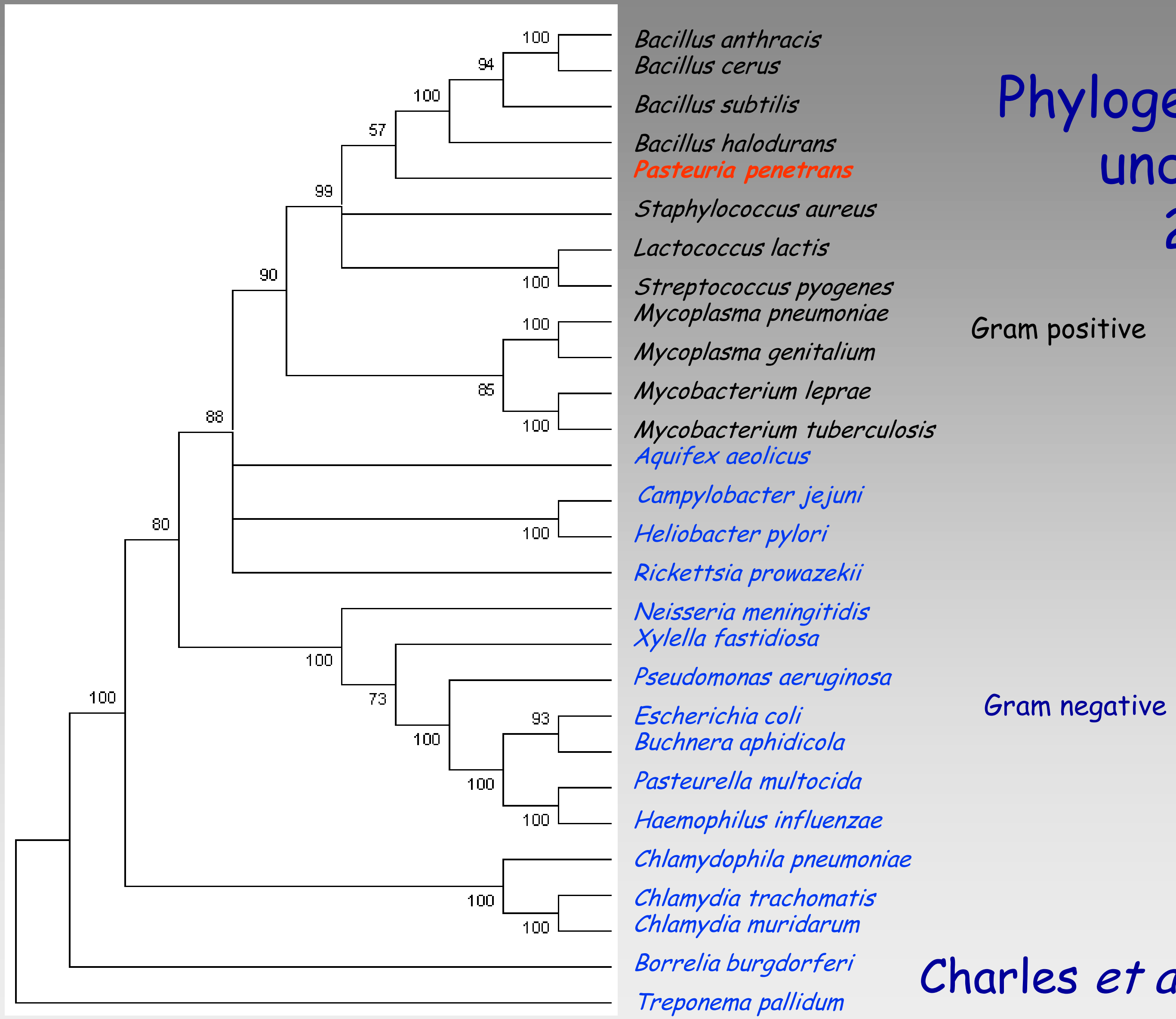
	Sanger-2003	454-2008
Sequence	2.5 Mbp	8.6 Mbp
Contigs	1500	5964 (782 > 2 kb)
Largest contig	2.5 kb	54.7 kb
GC content	62 %	45 %



Bacterial genomes range from:

< 1 Mbp *Mycoplasma genitalium*
to
> 9 Mbp *Nostoc punctiforma*

Genomics



Phylogenetic analysis undertaken using 27 full-length genes

Charles *et al.*, 2005, *J. Bact.*

Two major problems prohibiting *Pasteuria penetrans* from being developed into a commercial product related to endospore production

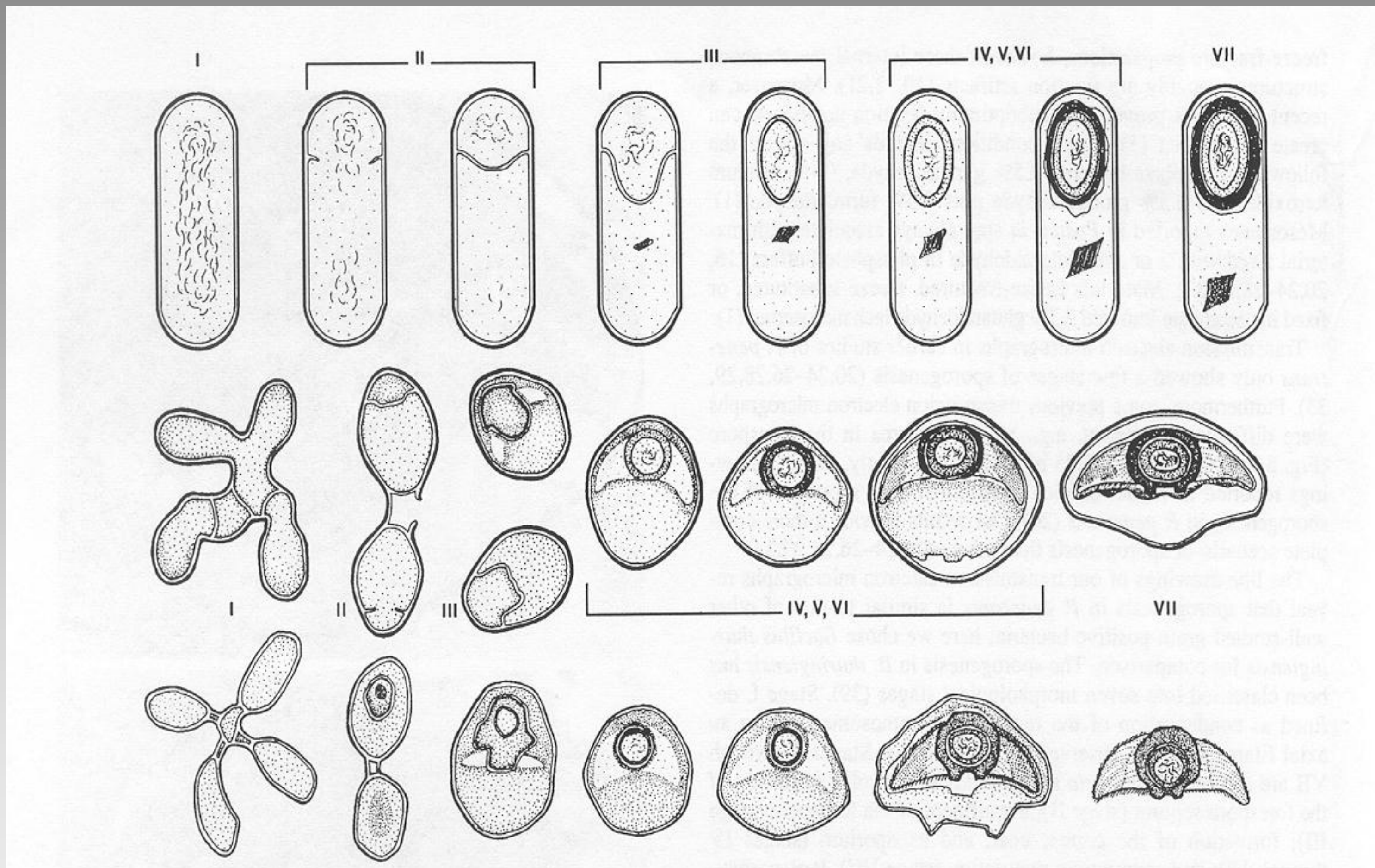
"Forget about the genome. And let's say, 'It's great. We've got all these sequences. Thank you very much for all the people that helped to get them - please get us some more.' And let's get on with the biology."

Sydney Brenner, January 2003, San Diego, California
Plant and Animal Genome Conference

1) In vitro mass production

2) Spore 'type' and attachment specificity

Comparison of endospore formation in *Bacillus thuringiensis* and *Pasteuria penetrans*

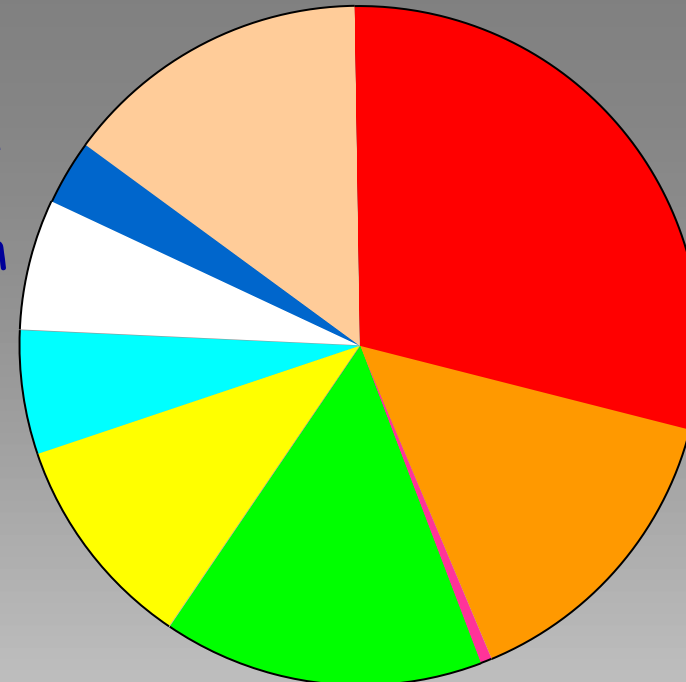


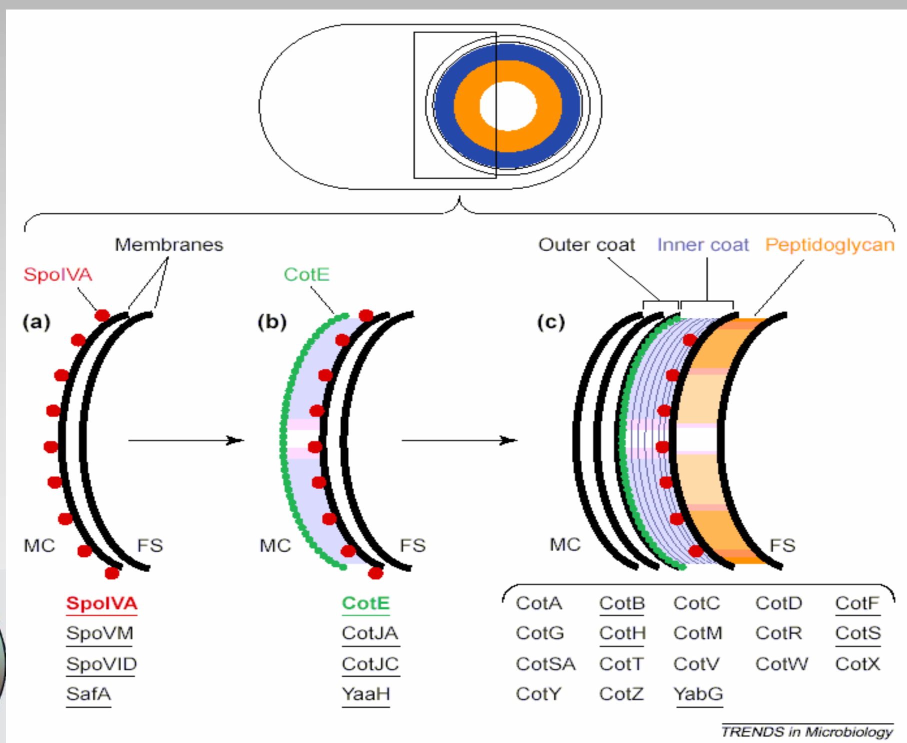
From Chen *et al.*, (1997) *Phytopathology* 87, 273-283

Genomics

GO analysis

- Sm all-molecule metabolism
- Broad regulatory functions
- Macromolecular metabolism
- Cell processes
- 6 -7 % → Sporulation
- Transposons
- Other
- Conserved hypothetical
- Unknown function





***Sporulation governed by* Spo0F**

Driks, 2002
Trends Microbiol. 10: 251

Phosphorelay pathway

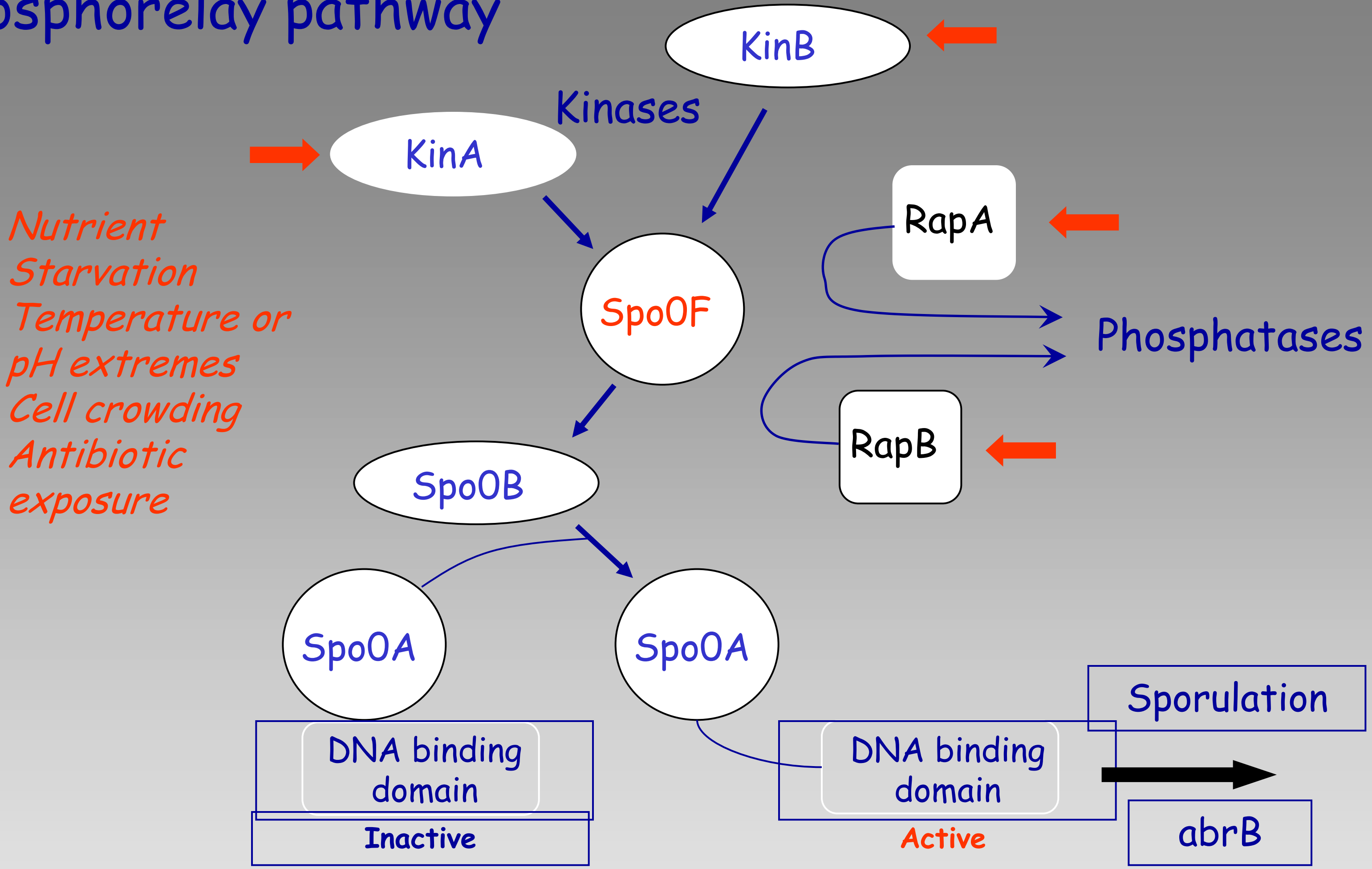


Diagram of proteins involved in the phosphorelay required for initiating the sporulation signal transduction pathway in *B. subtilis* (from Feher *et al.*, 1997). The red arrows represent environmental signals which initiate the transfer of the phosphoryl group via the Phosphorelay pathway.

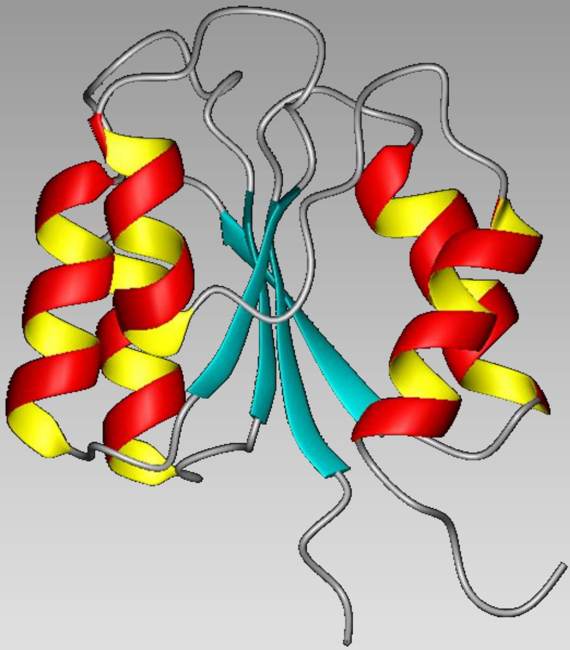
spo0F, Pasteuria and *Bacillus* species

```
                        ↓↓                                         ↓
Bsu  MMNEK--ILIVDDQYGIRILLNEVFNKEGYQTFQAANGLQALDIVTKERPDLVLLDMKIPGMDG
Ban  VMEGK--ILIVDDQYGIRVLLHEVFQKEGYQTFQAANGFQALDIVKKDNPDLVVLDMKIPGMDG
Bha  -MN-K--ILVVDDQYGIRVLLNEILQKDGYQMFQAANGIQALAIVEEETPDLVLLDMKIPGMDG
Ppe  MMNEKKLILIVDDQYAIRLLLKEIFSQDGIIVLQAAGGXEAIELVAQQQPDLMLLDMKMPGMDG

     ___________    ______________  ____  ____________  _____     __
         β1               α1         β2        α2        β3

                                    ↓                      ↓
Bsu  IEILKRMKVIDENIRVIIMTAYGELDMIQESKELGALTHFAKPFDIDEIRDAVKKYLPLKSN
Ban  IEILKHVKEIDESIKVILMTAYGELDMIQEAKDLGALMHFAKPFDIDEIRQAVRNELAVEA-
Bha  LEILRRIKDMNPNIEVIMMTAYGELNMINEAMQLGAVTHFAKPFDIDDVRAVIAENMKSS--
Ppe  LEILRRVRQVIPALKVIVMTAYGELEMMEKIRSLGVVMHFPKPFDVRAVRCGVLRYLXXXXX

     _________     ______     ________    ____   ______________
         α3          β4          α4        β5          α5
```

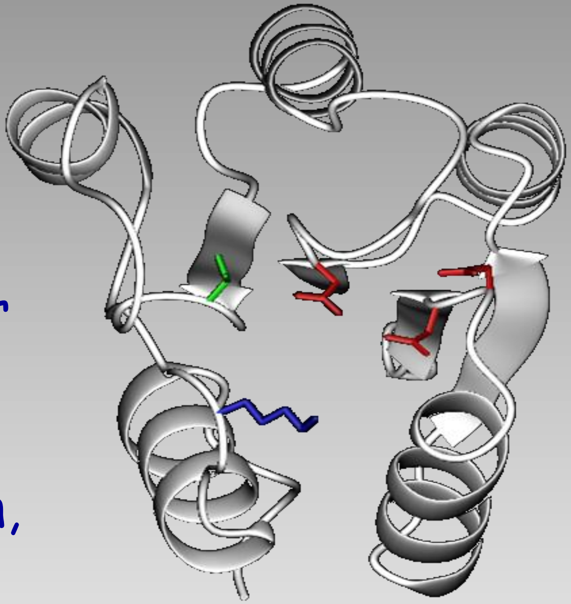
Residues in red are identical between *Ppe* and the *Bacillus* species. Residues in green are conservative exterior substitutions. The positions of b-strands and a-helices are indicated. Conserved active-site residues are highlighted in bold (arrows).

Kojetin, *et al.*, 2005, *Biometals* 18, 449-466

STRUCTURE OF Spo0F charged aspartic acids line the pocket of the active site



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



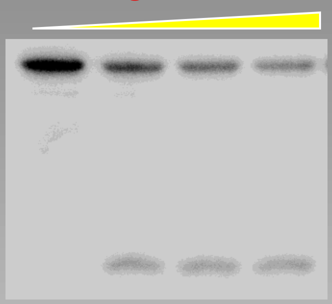
WHAT ABOUT OTHER IONS?

Kojetin, *et al.*, 2005, *Biometals* 18, 449-466

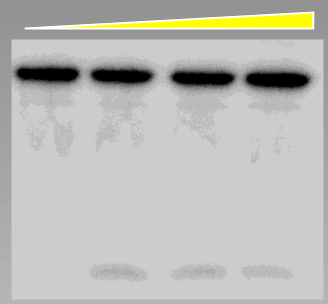
Divalent Metal Dependent Phosphorylation

Phospho-transfer from KinA~P to Spo0F

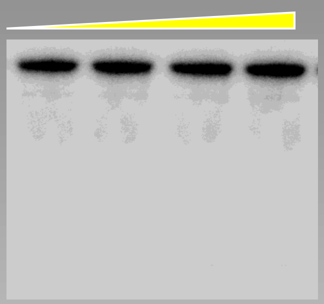
$MgCl_2$



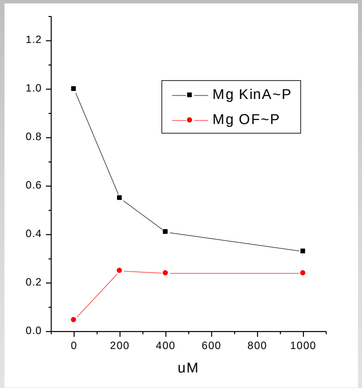
$ZnCl_2$

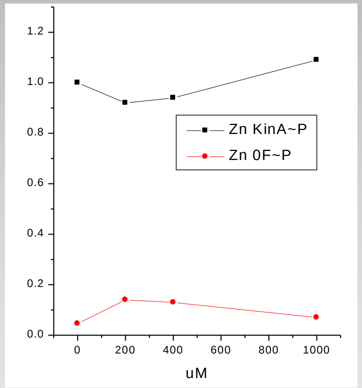


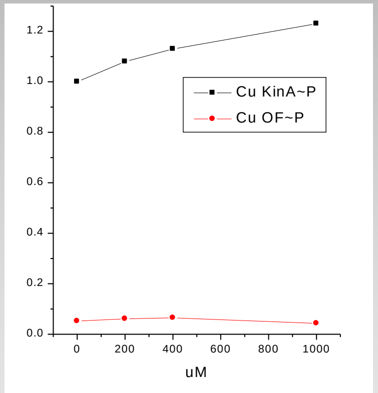
$CuCl_2$



Relative amount of KinA~P or Spo0F~P



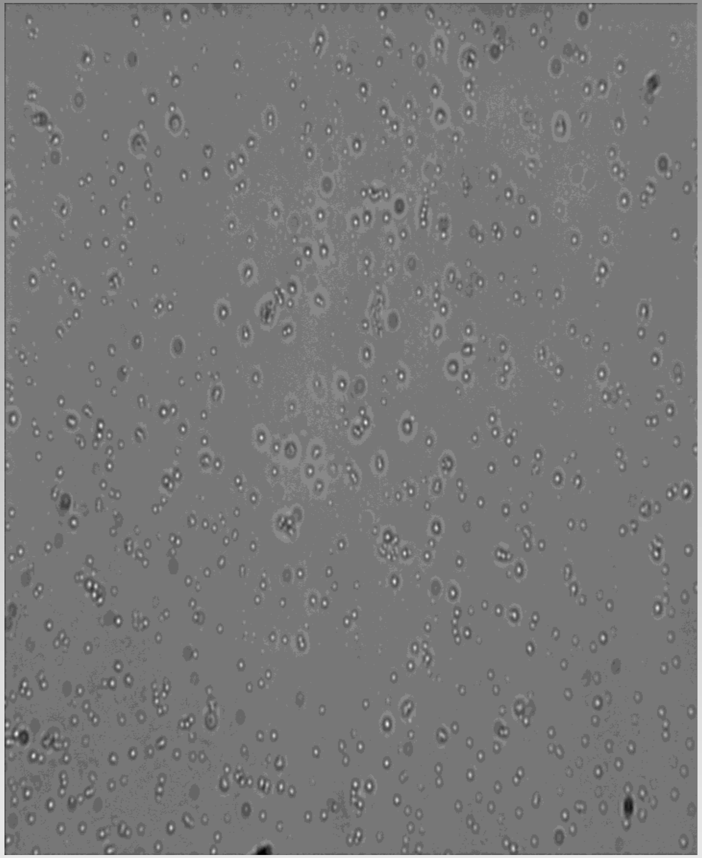




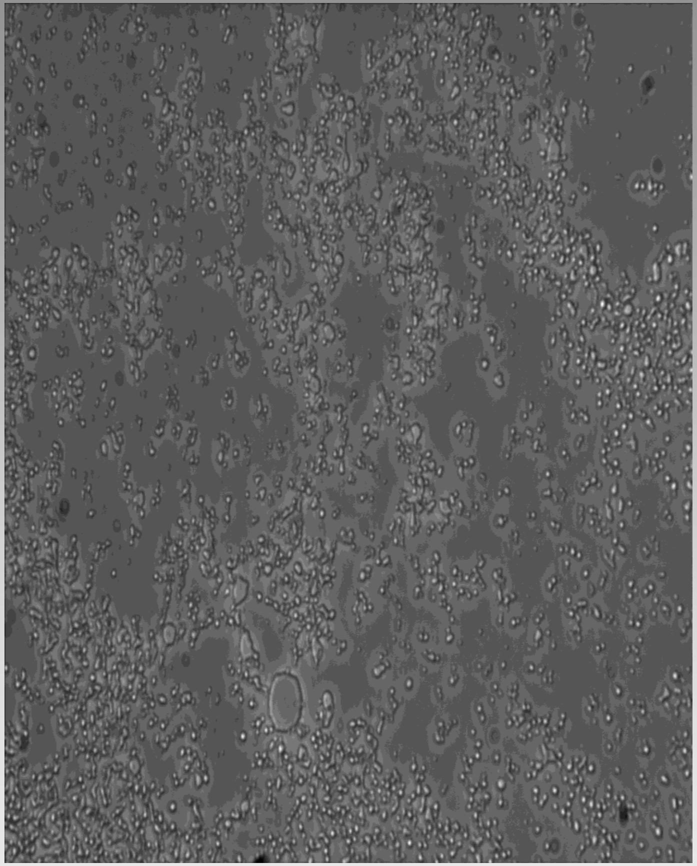
KinA~P Spo0F~P

Kojetin, *et al.*, 2005, *Biometals* 18, 449-466

Growth of *Pasteuria* in culture is affected by divalent cations



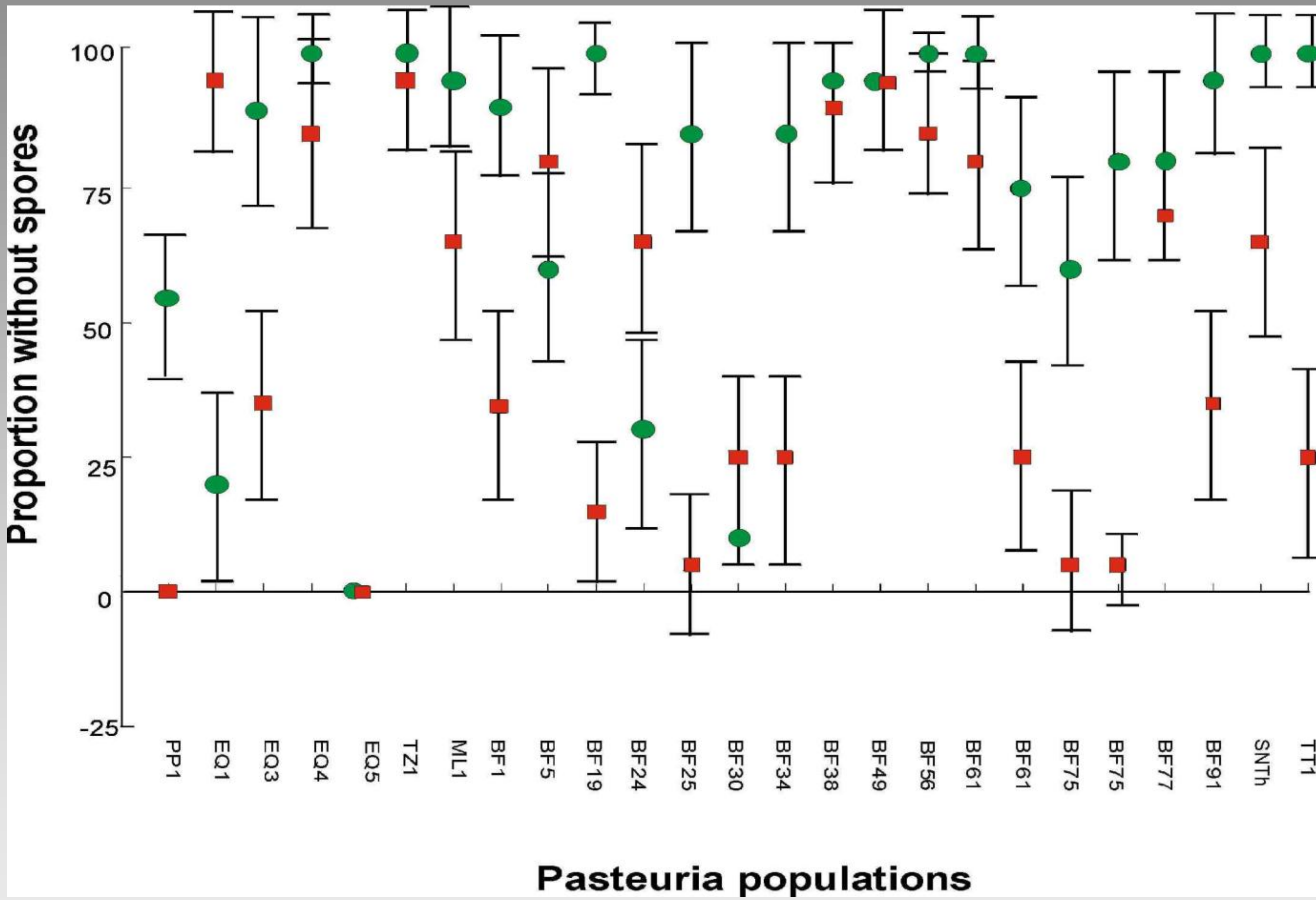
$+ Cu^{+2}$



$- Cu^{+2}$

Kojetin, *et al.*, 2005, *Biometals* 18, 449-466

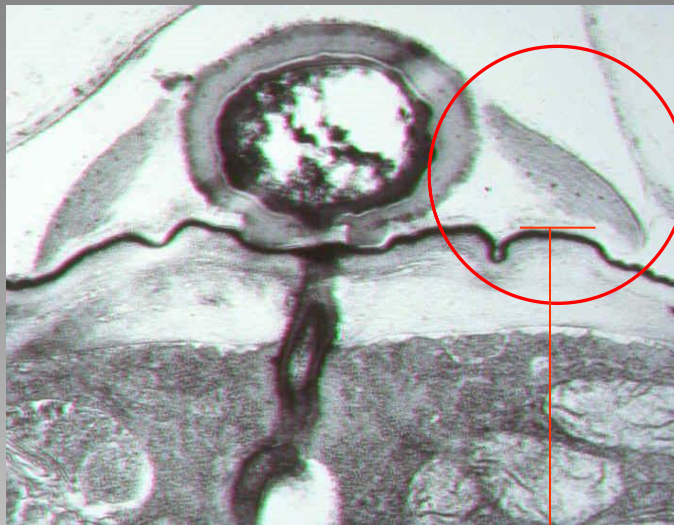
Percentage of juveniles of *Meloidogyne arenaria* ● and *M. incognita* ■ to which no spores attached

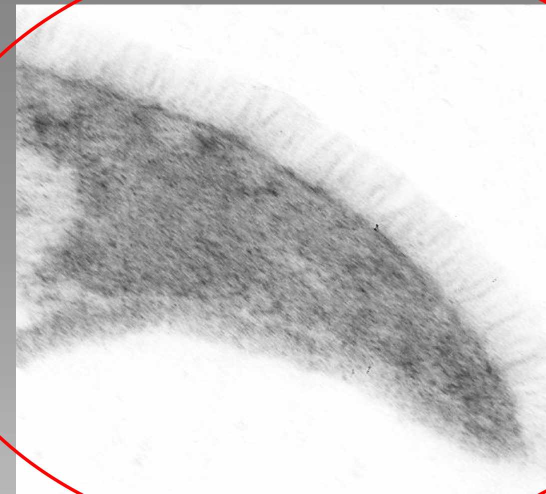


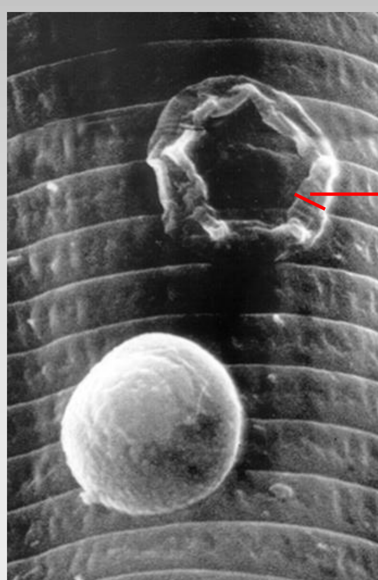
Davies *et al.*, 2001, *Parasitology* 122, 111-120

Attachment is a key determinant for infection

Pasteuria penetans
endospore





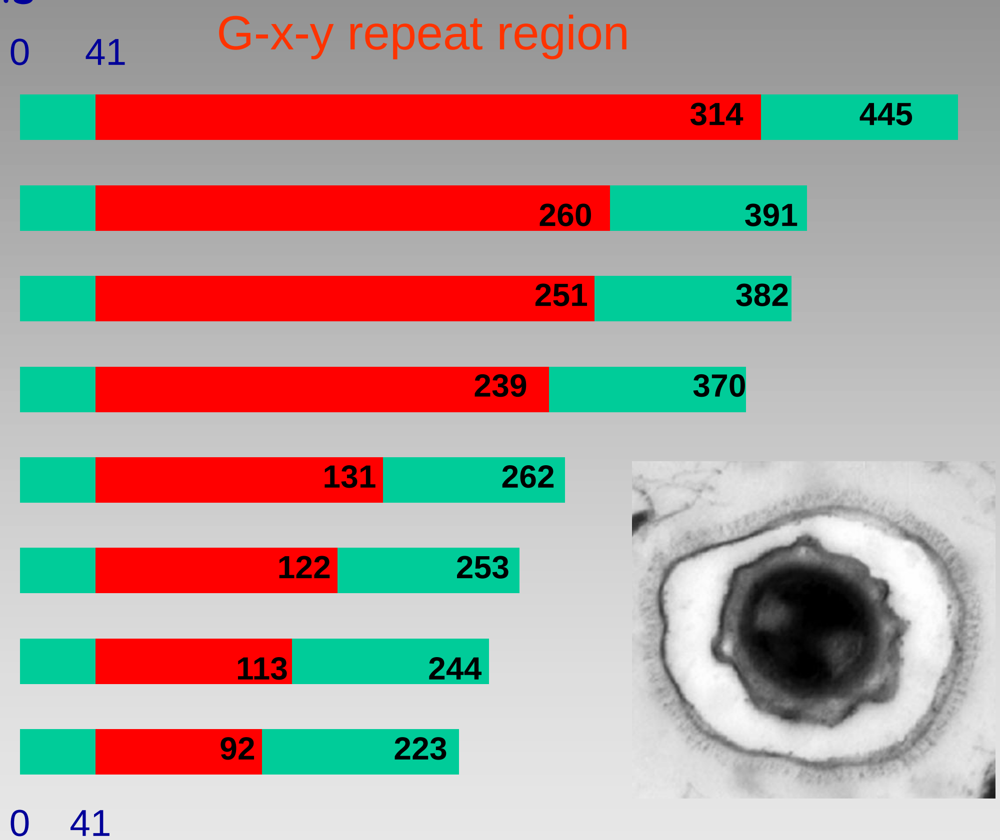


Bacillus anthracis
endospore

Davies, *Advances in Parasitol. In prep.*

GENOMICS & ENDOSPORE ATTACHMENT

B. Anthracis Strain	Number G-x-y repeats	Filament length (nm)
770	162	60.8
818	130	47.3
Ames	124	NA
53169	118	48.7
9602R, A2R, 6183R	52	28.4
6602	48	29.8
5725R	42	25.1
4229	28	13.9

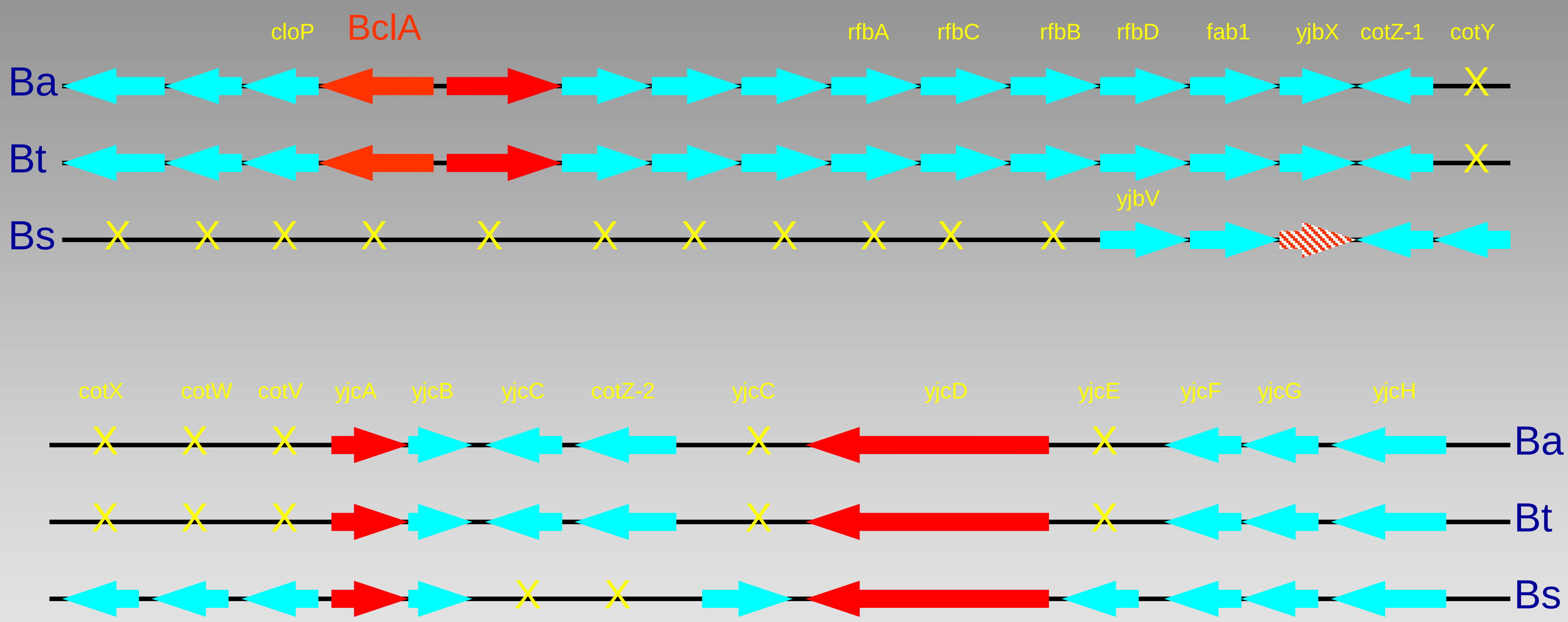


Structure of BclA collagen-like repeats and their relationship to filament length in *Bacillus anthracis*

(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*

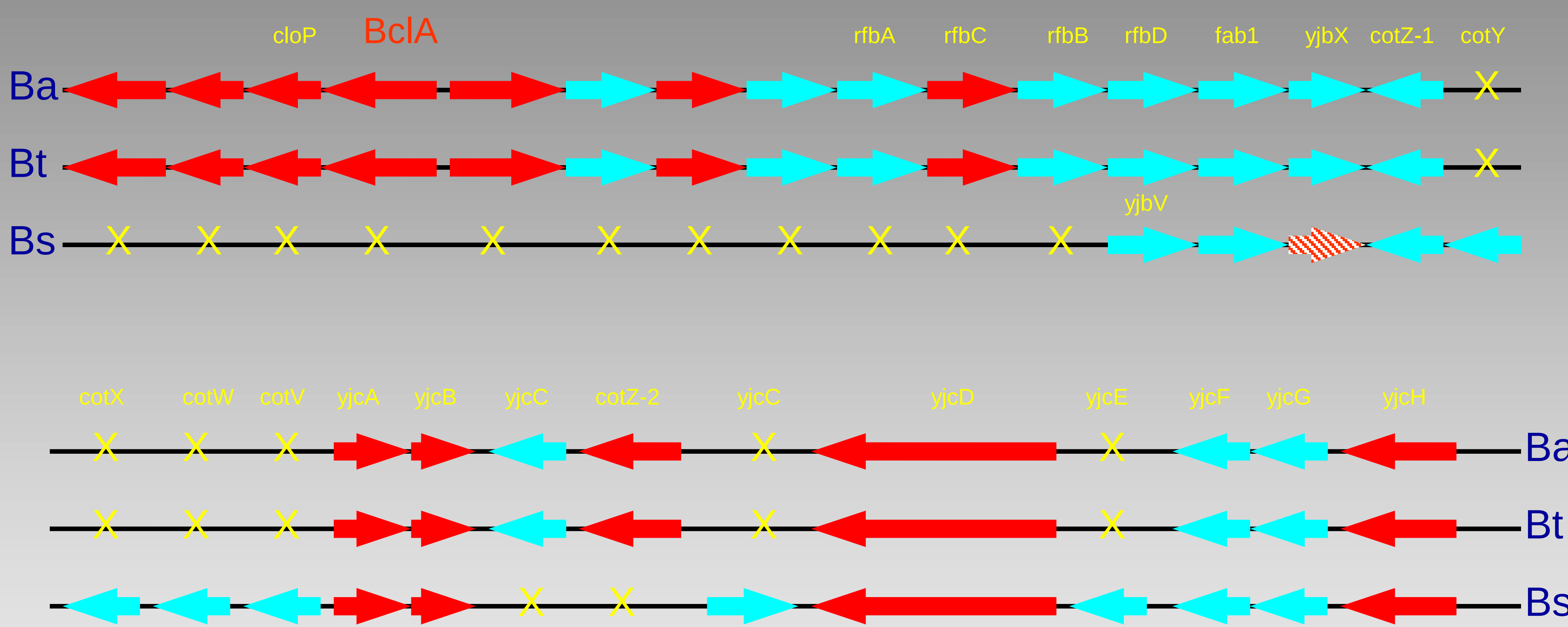


Solid red genes denote BLAST hits (e^{-14}) to *Pasteuria* (dashed red e^{-4})

(Davies & Opperman, *IOBC Bulletin*, 2006,)

COMPARATIVE GENOMICS as of 2008

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 (Ppenetrans-147)

IFLGTTIGRLEFSGREFALGREGQQEHRGGPTTGPPGPPGAHGIQGPPGAQGIQGPAGTP
GAQGIQGPPGPAGTPGAQGIQGPPGPAGPAGPAGAAGSPGTPGPAGPAGPAGAAGSPGTP
GSPGTPGPAGPAGPAGPAGTPGTPGSPGTPGPAGPAGPAGTPGTPGAAGSPGTPGPAGPA
GPAGPAGTPGTPGPAGPQGTPGAPGPAGPQGTPGAPGPAGPQGTTGAAGPTGPQGTTGPQ
GTQGTQGPQGIQGIQGPVGPQGATGATGPGLNTSMTIVAGGGADTQFITPTPEGATGTAV
TLETGNGQRYGSGDIQLIGTTDILLPSTGTYLMSFHIDANYTSAAGAPVAGAYGSYVAYF
RQFTTDFFFNQIVAFWVGPALVNDAFDSSISNTVLGCVSDIPPHGLNNHMVRHESDVANS
LNTRRSQPQX

79 G-X-Y repeats

>Contig2_1 (Ppenetrans-147)

LQDQTNSPFRLEPEMWSINNIHSFNHMNKRKKQNIFFHTFSLGGLEDNHFMKHWIGRNSG
CIHYKNNGKIRNTITHTPSPRRSEGNRFVKHWIGRKSVYINSDYRDQHNHHNSSRTTLYR
NCEKCDNNQYEEFDNDHCEEFDNNHCCDCCLCNRCKCRVTGPTGPTGPTGRTGSTGRTGP
TGPTGRTGSTGRTGPTGPTGPTGPTGPTGRTGFTGRTGSTGSTGRTGPTGSTGRTGSTGS
TGRTGPTGSTGRTGSTGSTGRTGSTGSTSRPLVGRRNSR

36 G-X-Y repeats

>Contig3_4 (Ppenetrans-147)

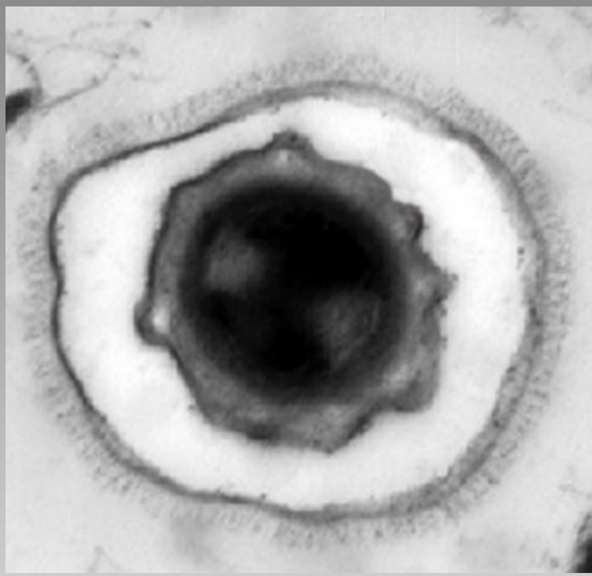
VANSRLEGWTAGPAGAQGISGPPGEPGIQGPAGTPGAQGIQGPPGPAGTPGAQGIQGPPG
PAGPTGPAGAAGSPGTPGPAGPAGPAGAAGSPGTPGSPGTPGPAGPAGPAGPAGTPGAPG
PAGPQGTPGAPGPAGPQGTPGAPGPAGPQGTTGAAGPTGPQGTTGPQGTQGTQGPQGIQG
IQGPVGPQGATGATGPGLNTSMTIVAGGGADTQFITPTPEGPTGPGGTFEPGNGPKYREG
GELHLIGTHRFSSSRVPGPF

62 G-X-Y repeats

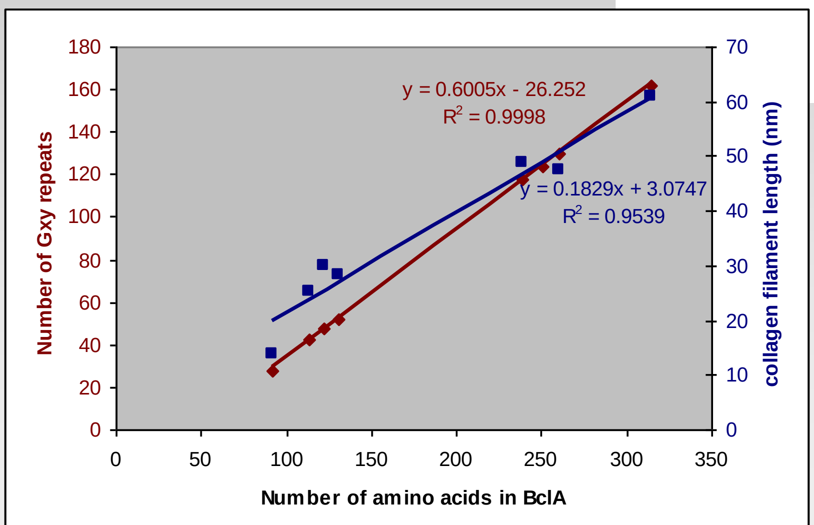
Total of 12 *Pasteuria penetrans* collagens with G-X-Y repeats

5 unique to RES147, 4 unique toFl-1, 3 in common

GENOMICS & ENDOSPORE ATTACHMENT



Pasteuria Contig	G-x-ys	upstream	downstream	Amino acids	Filament length (nm)
Con 1_2	79	Yes	Yes	430	81.7
Con 374	35	Yes	Yes	221	43.5
Con 3_4	62	Yes	Yes	260	50.6

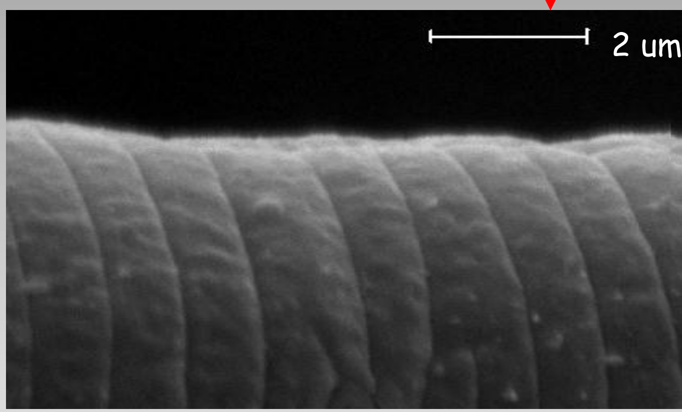


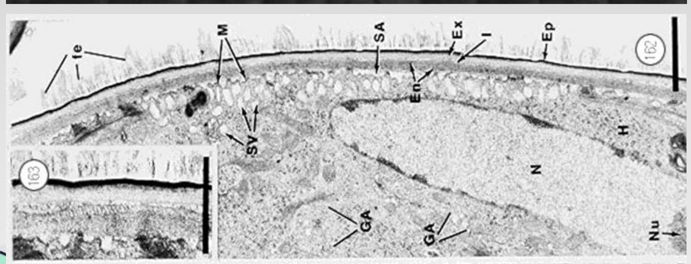
Prediction of *Pasteuria* filament length from the equation

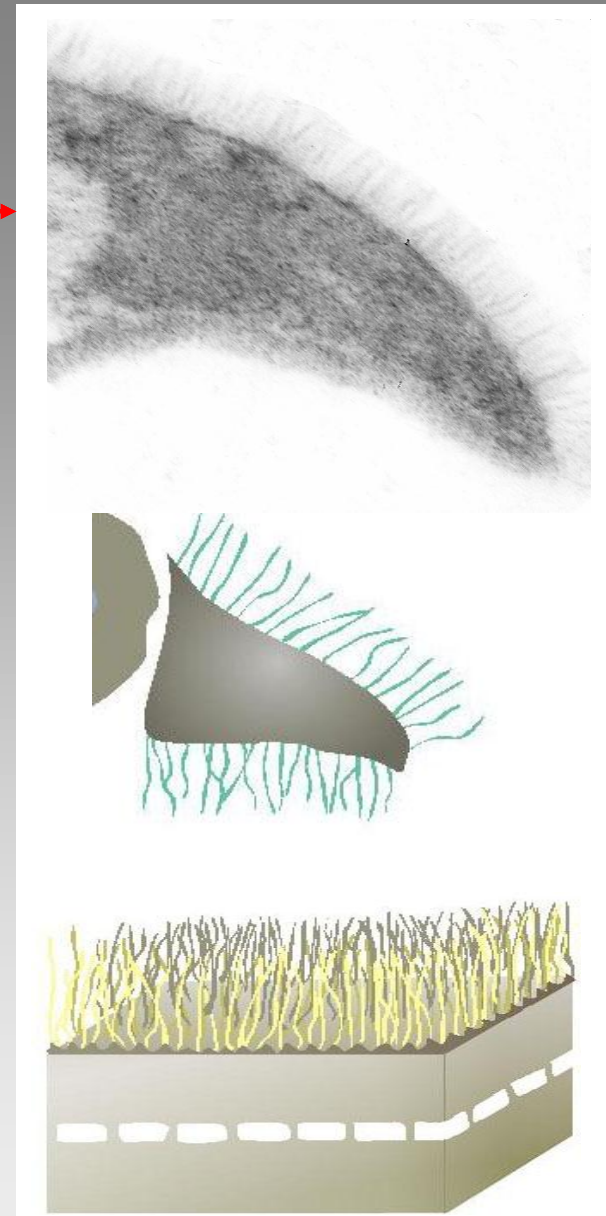
$$y = 0.1829x + 3.0747$$

(Davies & Opperman, *IOBC Bulletin*, 2006,)

Spore attachment: *Velcro*-like mechanism

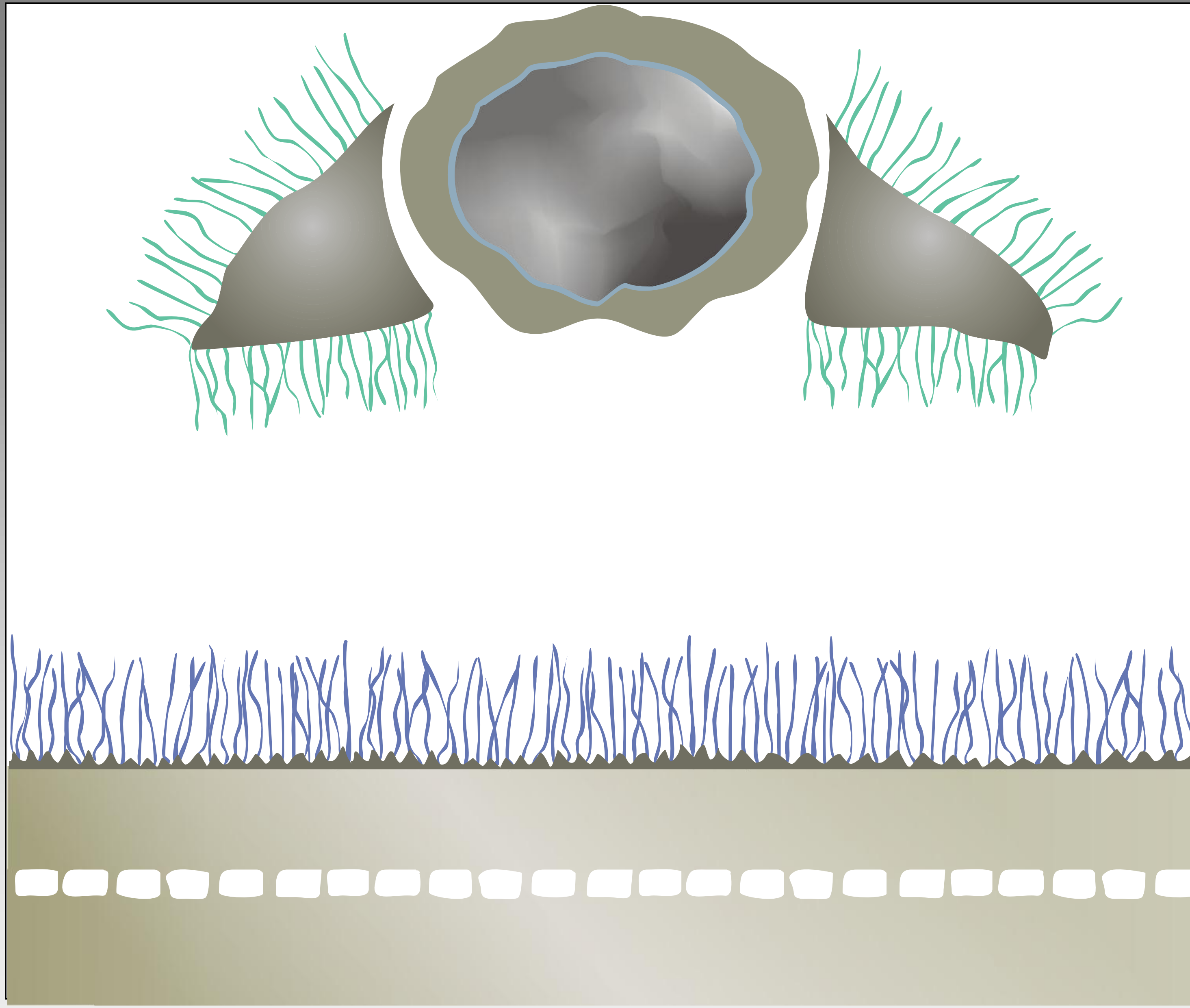






Davies, *Advances in Parasitology* In press.

Spore attachment: *Velcro*-like mechanism



Davies, 2008, *Advances in Parasitology* in prep.

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

Nematode spp.	Chromosome No.	Mode of reproduction
M. arenaria	30-46 polyploid	Mitotic parthenogenesis
M. incognita	41-46; polyploid	Mitotic parthenogenesis
M. javanica	42-48; polyploid	Mitotic parthenogenesis
M. hapla Race A	13-17; n	Amphimixis & meiotic parth.
M. hapla Race B	43-48; polyploid	Mitotic parthenogenesis

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

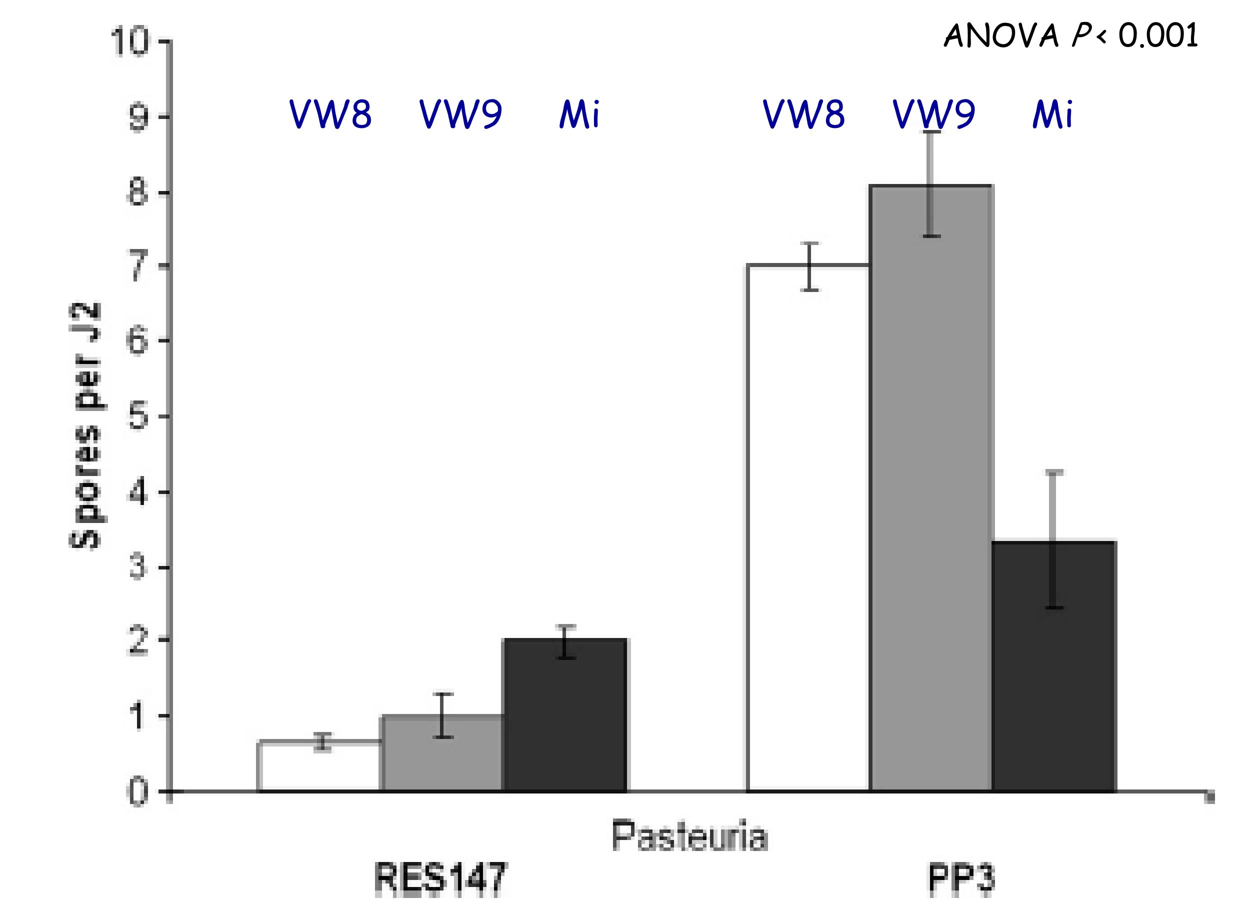
VW9 single juveniles

2 single egg masses from 3 single J2 populations

Tested against PP3 & RES147

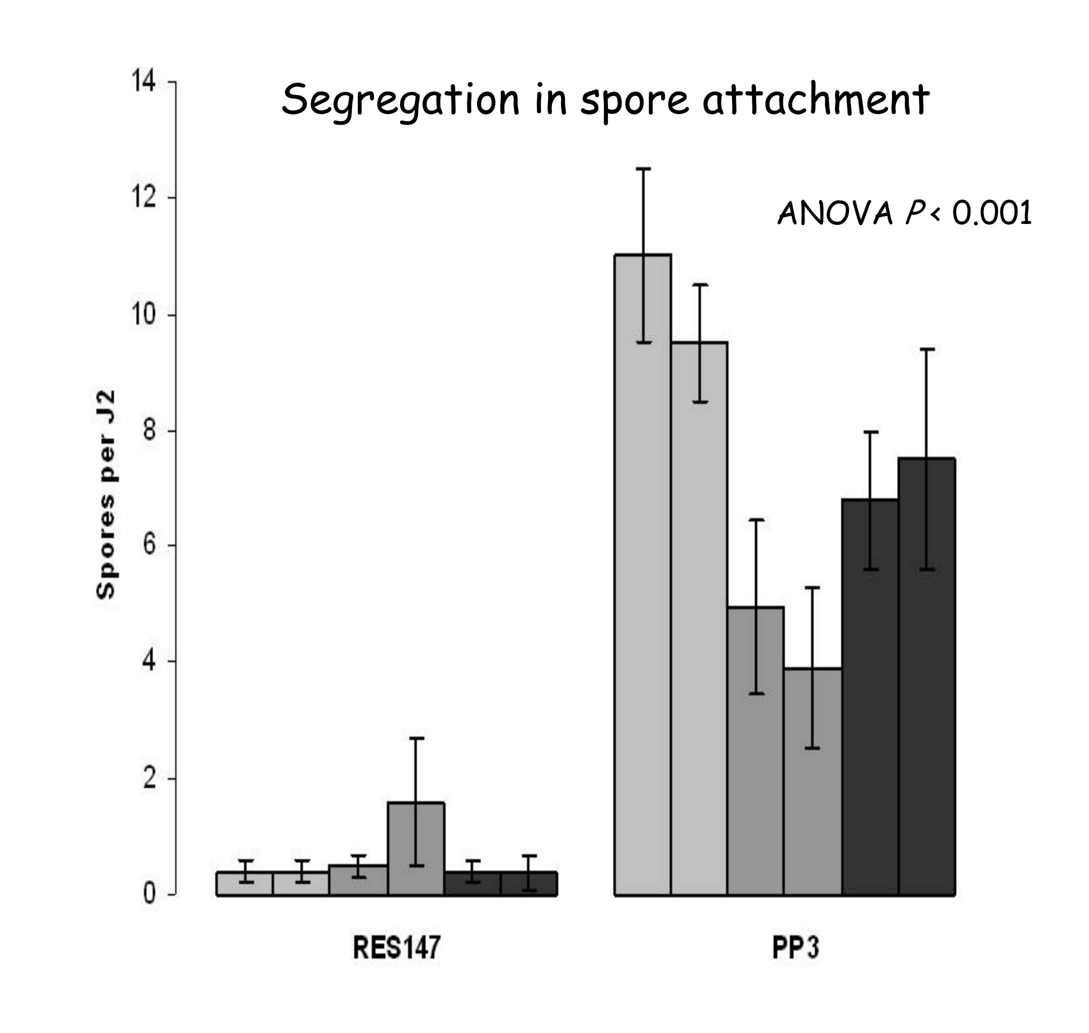
Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

Endospore attachment of *Pasteuria* populations RES147 & PP3 to *Meloidogyne hapla* strains VW8 & VW9 and *M. incognita*



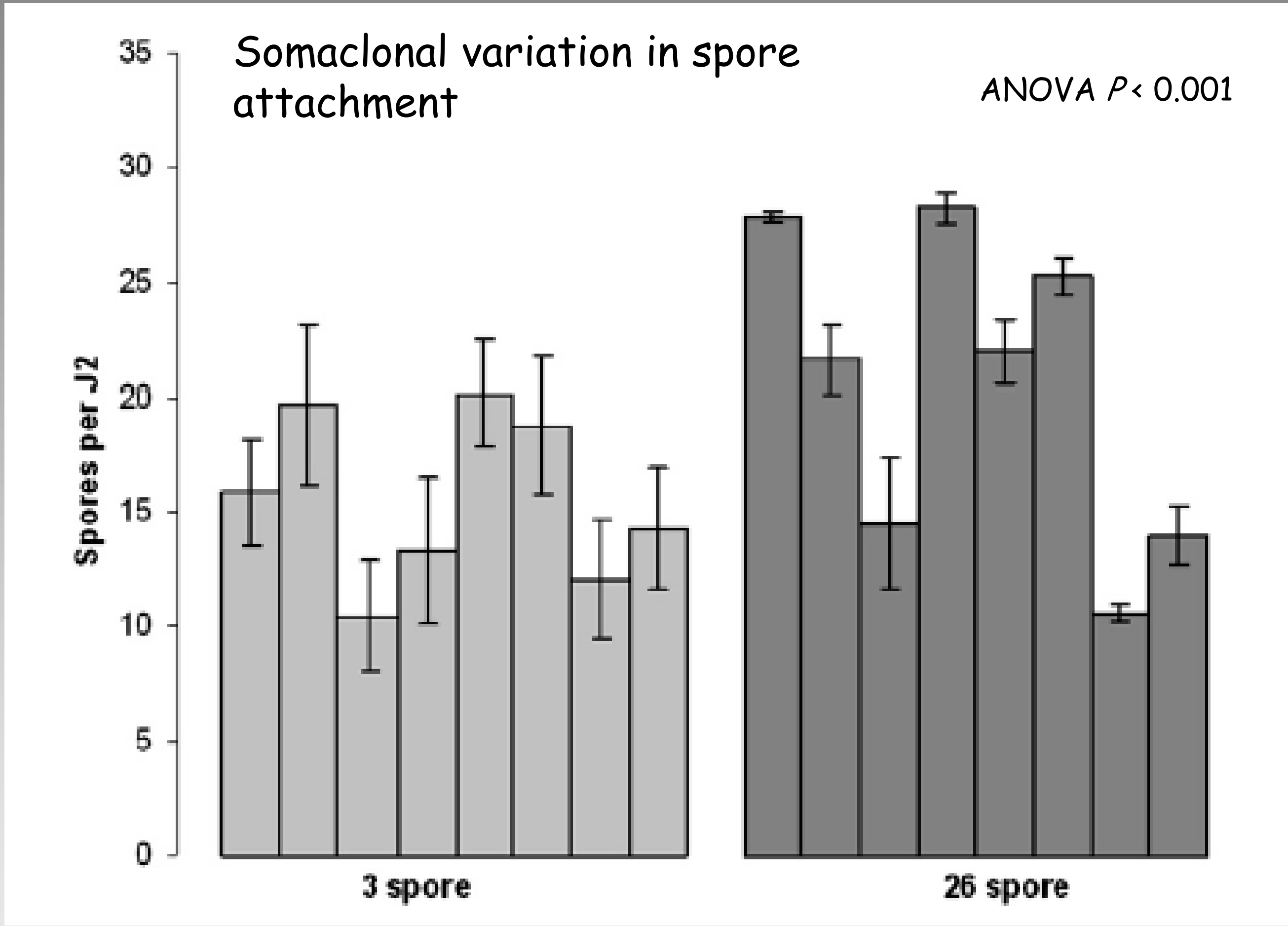
Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

Spores, RES147 & PP3, attaching to single juvenile descent lines arising of *M. hapla* VW9K1 (light grey), VW9K2 (dark grey), VW9K3 (black)



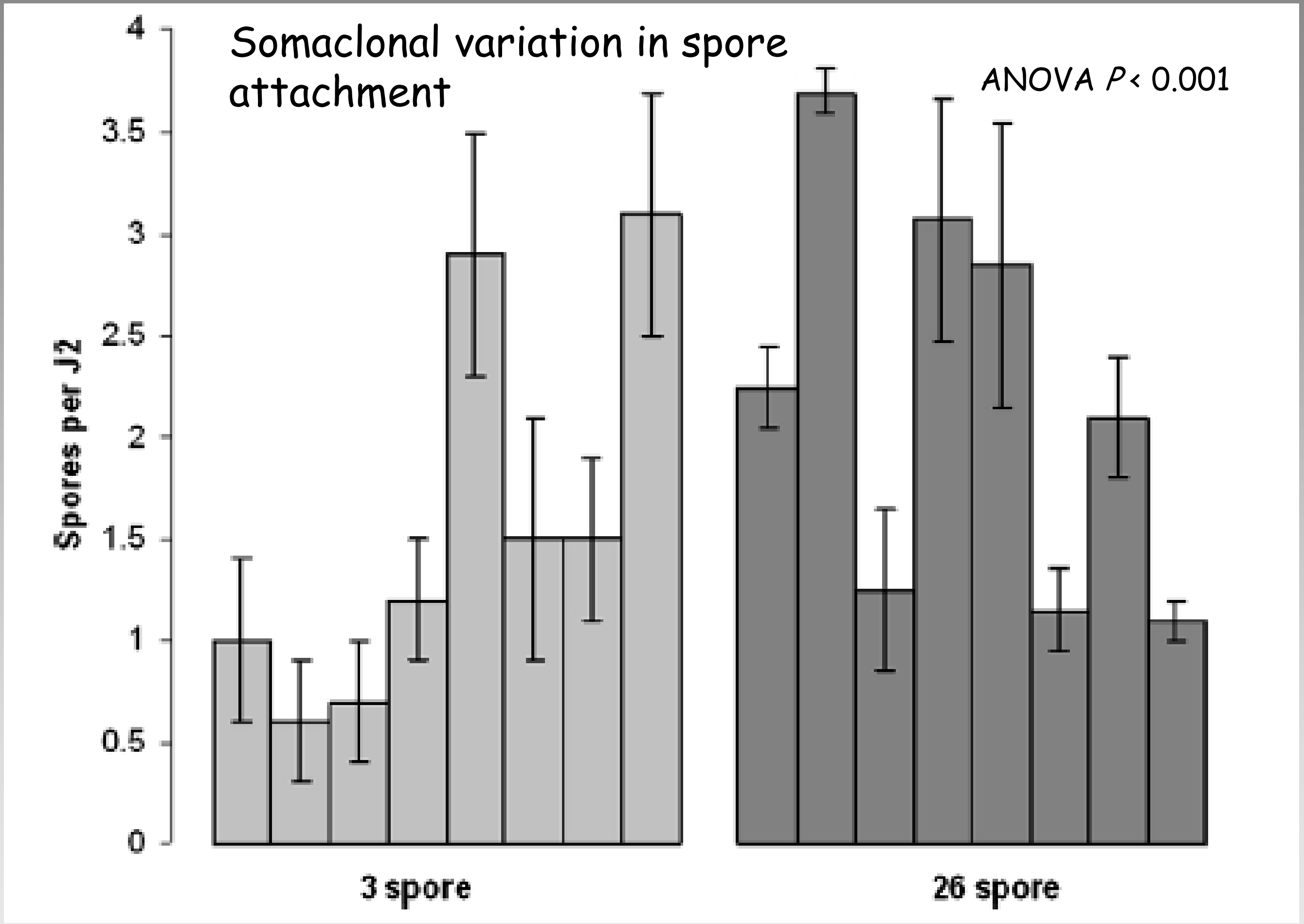
Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

Endospore attachment of *Pasteuria* population PP3 to single juvenile descent lines of *Meloidogyne incognita*



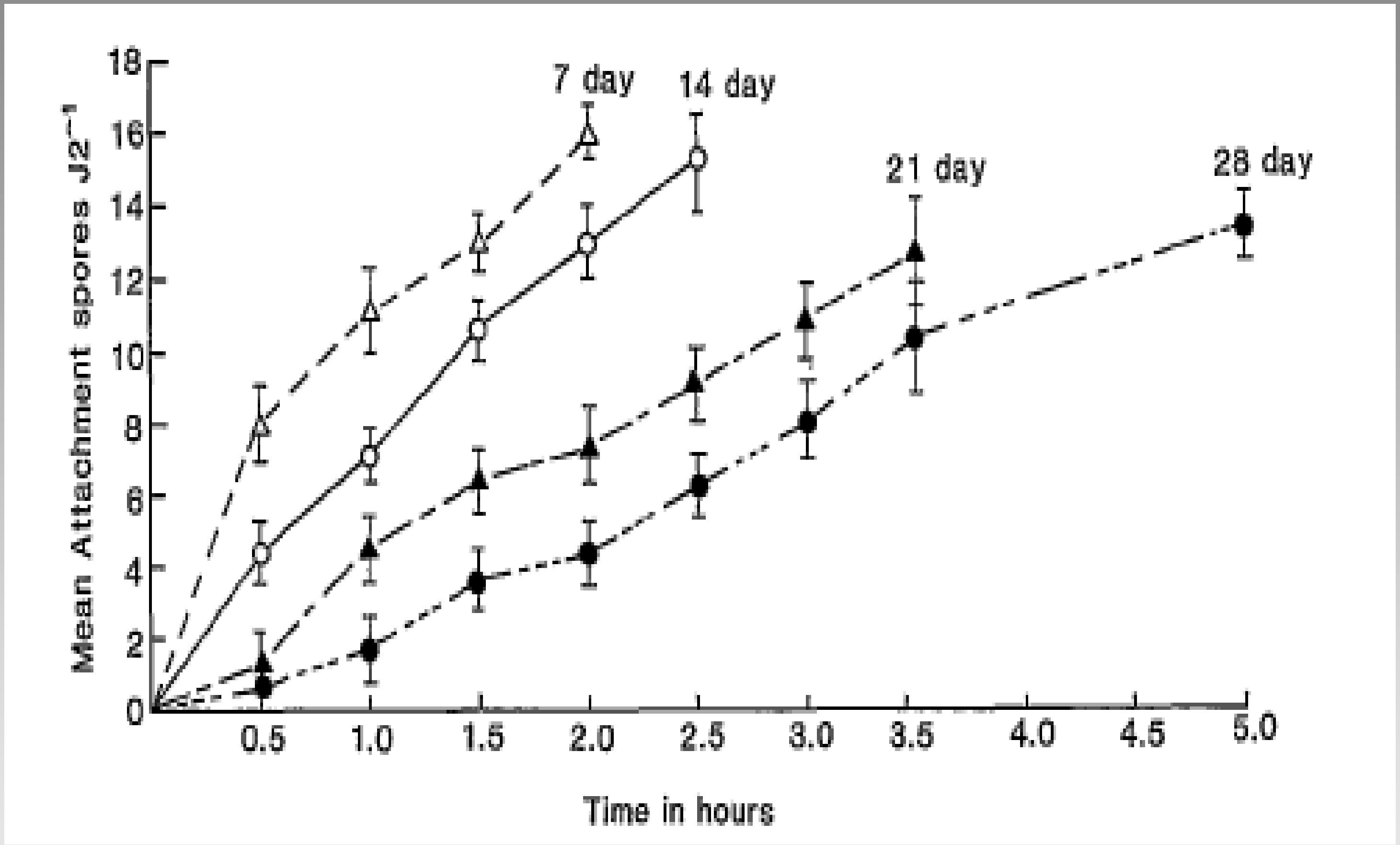
Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

Endospore attachment of *Pasteuria* population RES147 to *Meloidogyne incognita*



Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

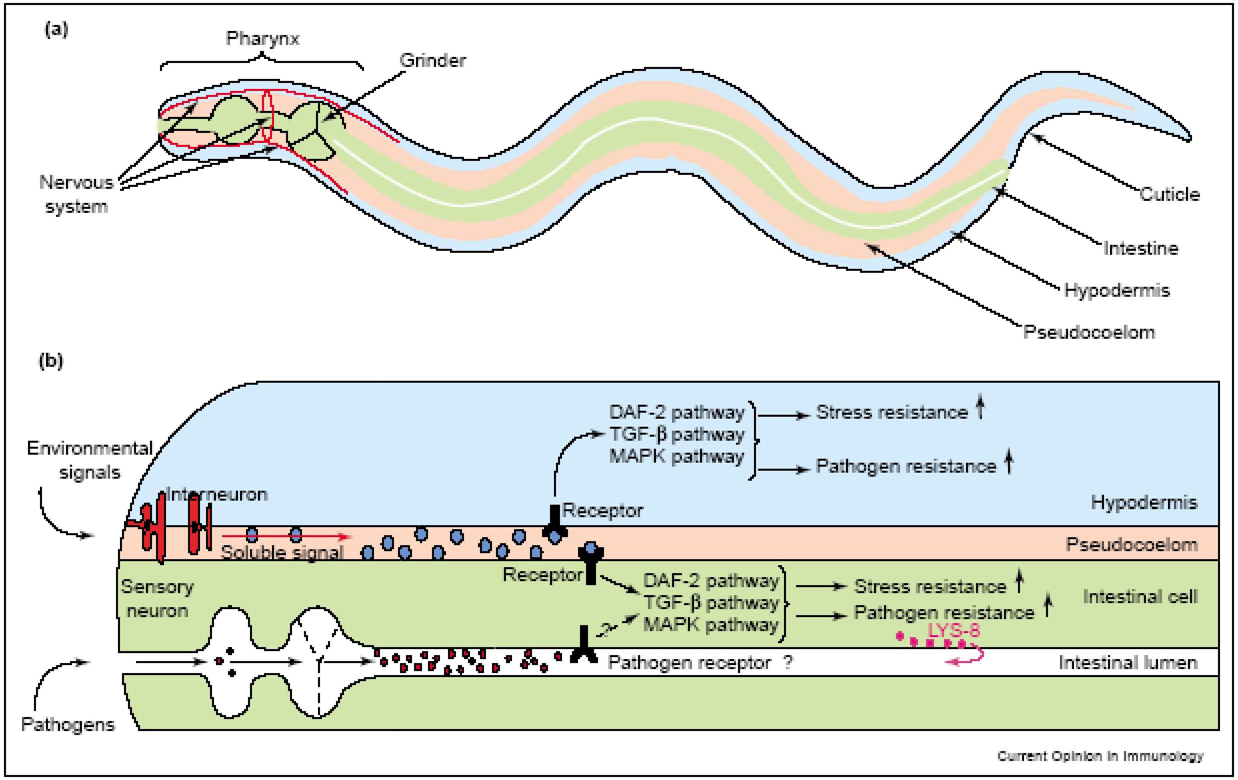
Relationship between juvenile age and spore attachment



Davies *et al.*, 1991 *Revue de Nematol* 14, 616 – 618.

DAF-2, IGF & innate immunity in *worms*

Figure 2



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*

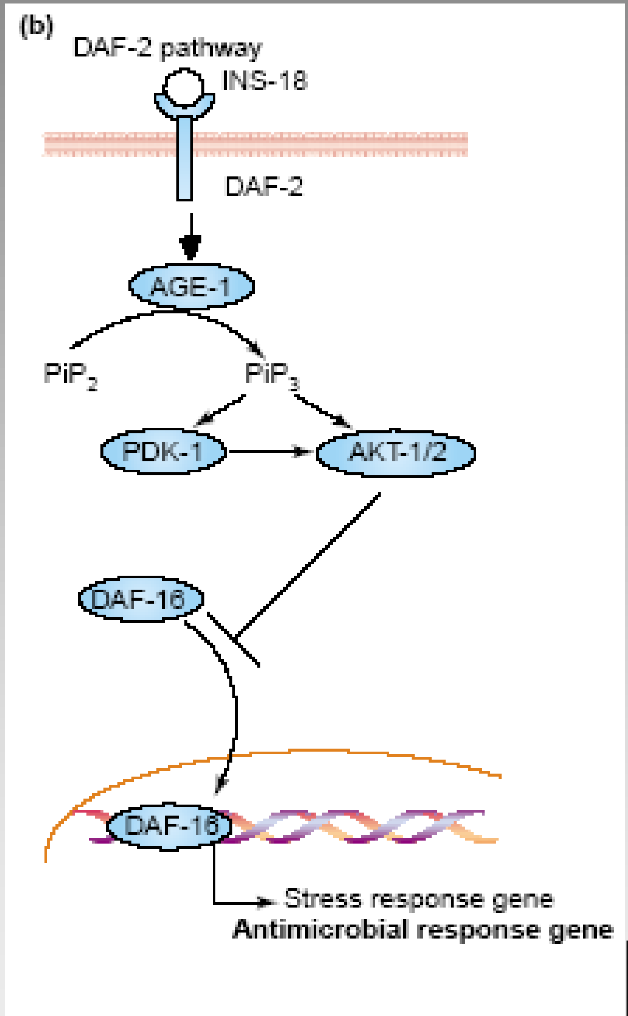
Activation of phosphorylation pathway begins with binding of insulin-like ligand to DAF-2 receptor (38 present in Ce only a few characterised)

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

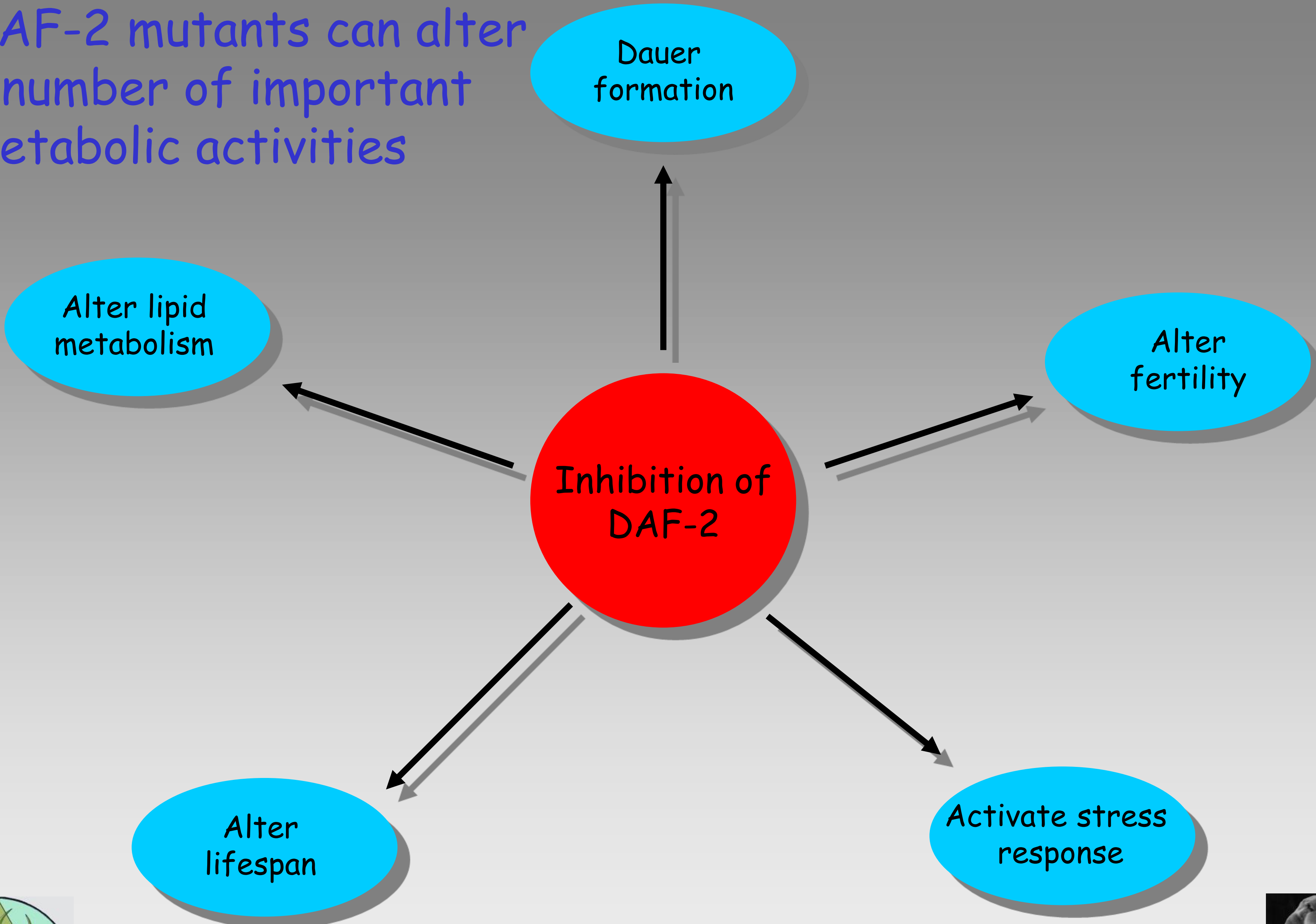
DAF-16+P cannot be translocated to nucleus to activate DAF-2 pathways

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

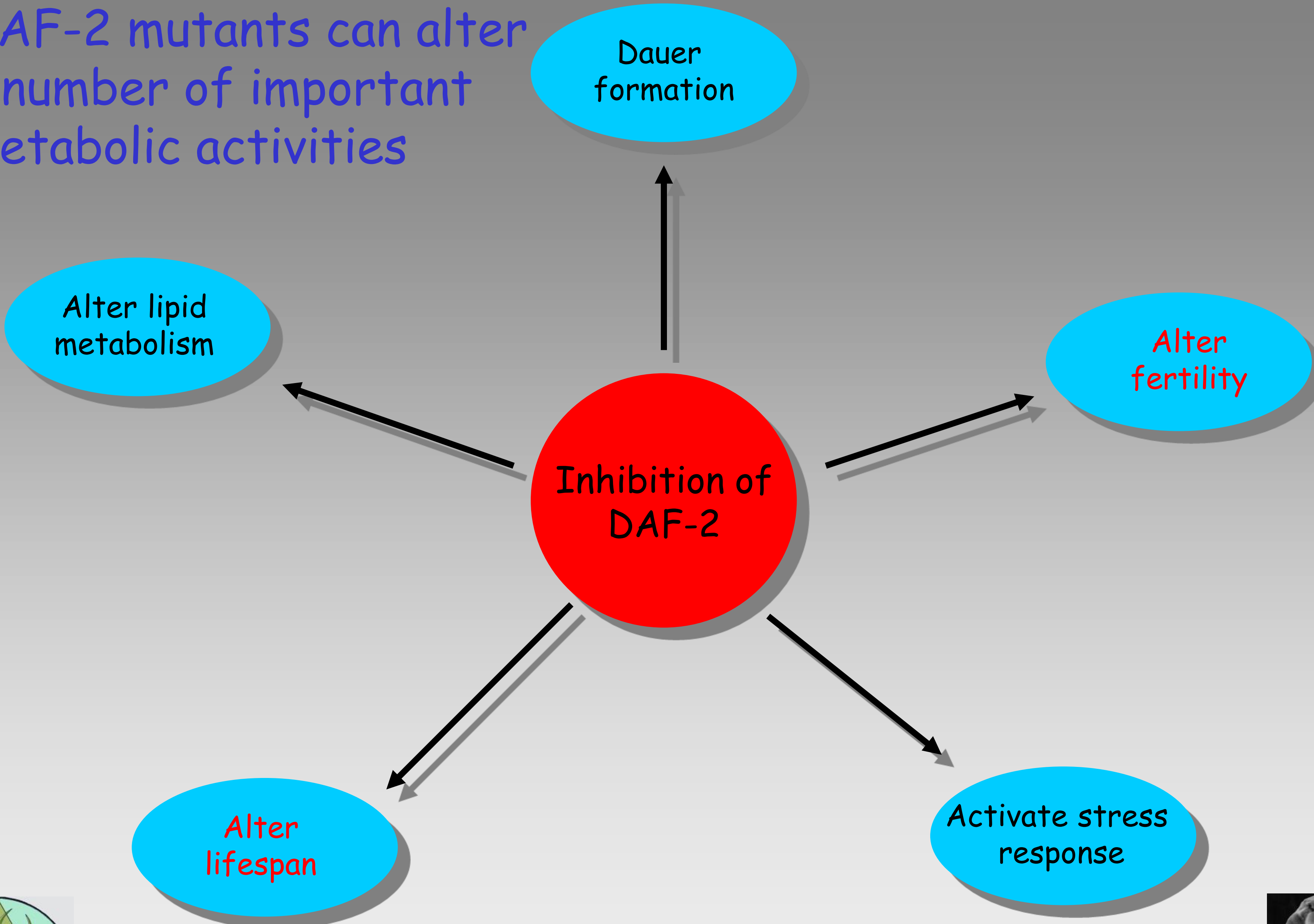


(Millet & Ewbank, 2004)

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



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

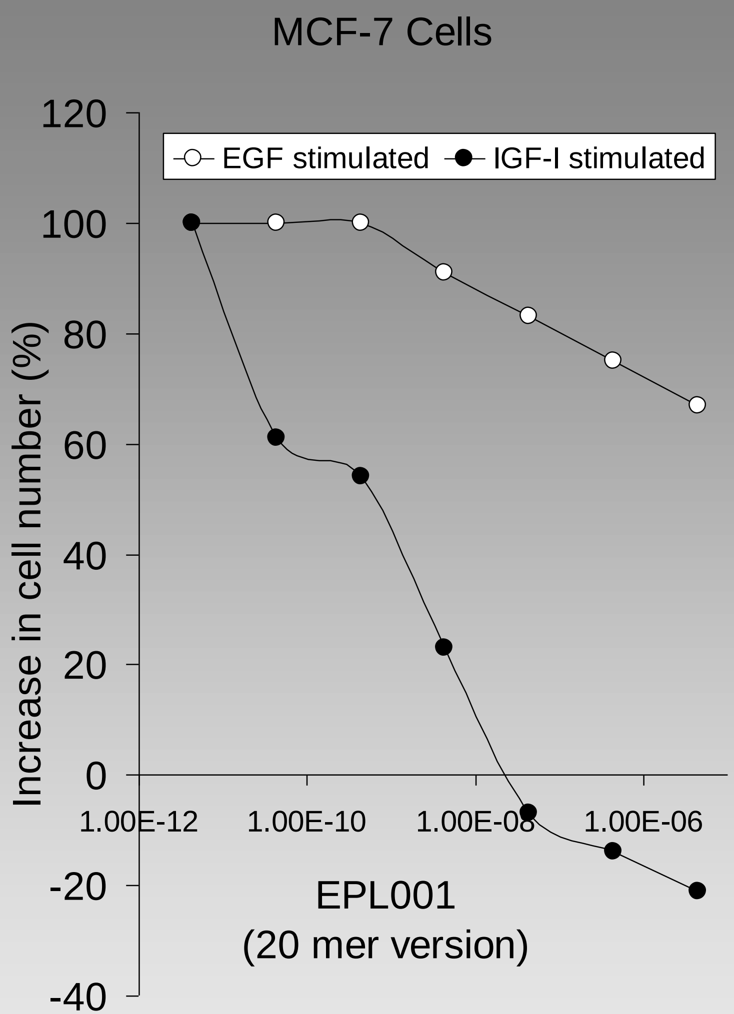


EPL001 is peptide that inhibits IGF:

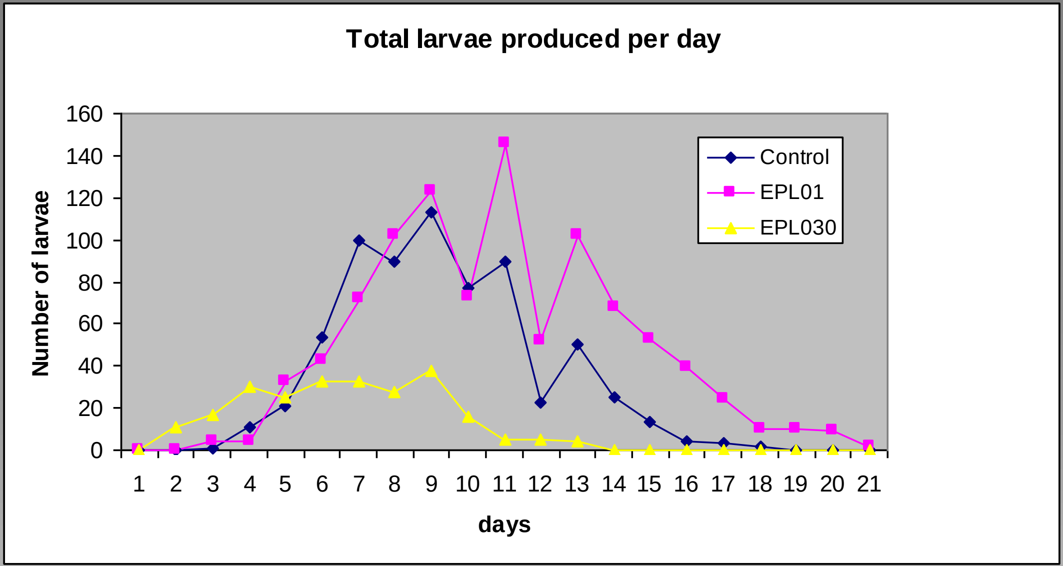
72 hours after application EPL001 inhibits

Epidermal Growth Factor (EGF)
&
Insulin Growth Factor (IGF)

stimulated
MCF-7 cells
(breast carcinoma cell line)



John Haylor *et al.*, in prep.

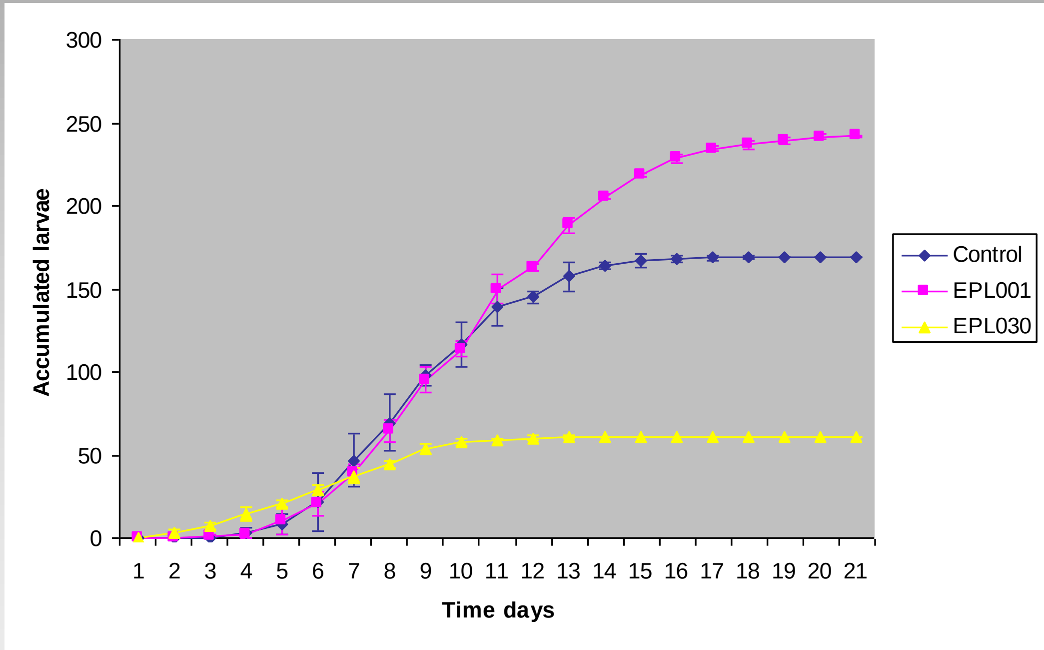


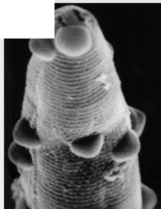
Manipulation of life-span and fecundity in *C. elegans*

Mean larvae per adult

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

ANOVA $P < 0.05$



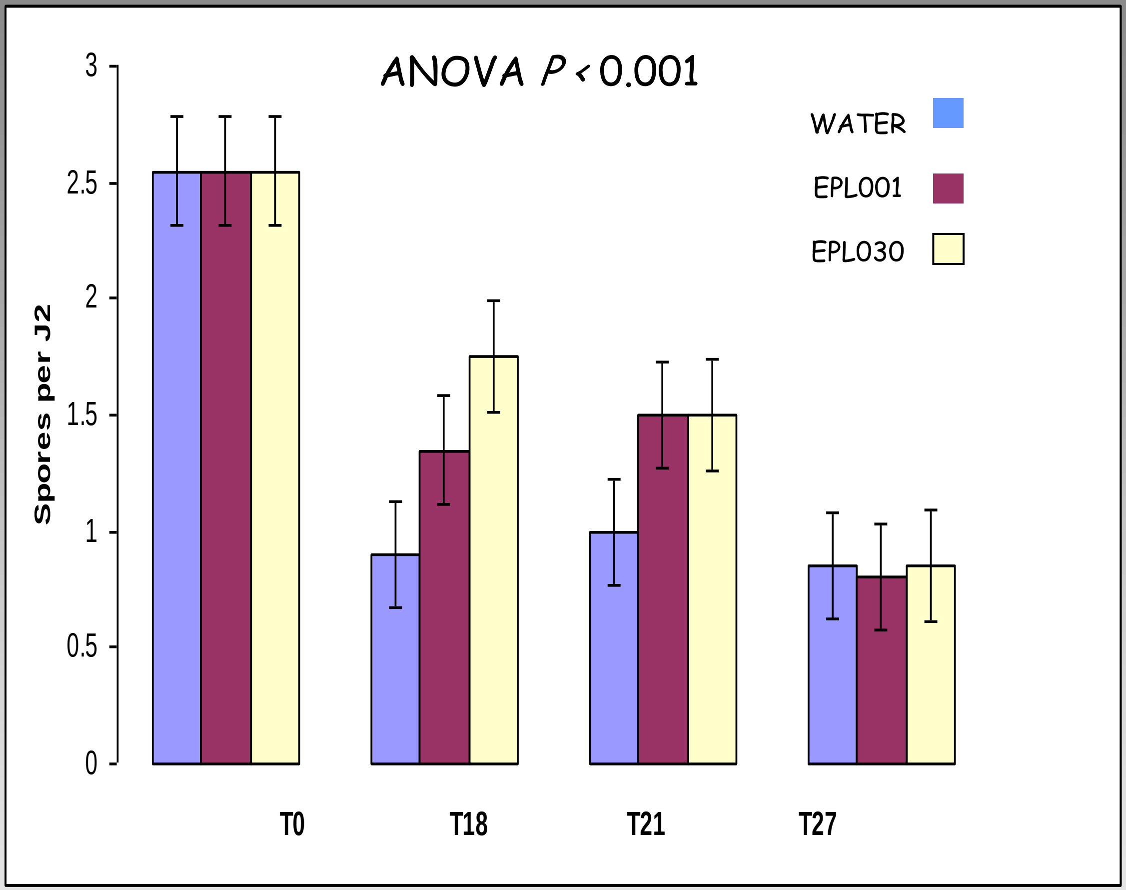


Davies and Hart (2008) *Nematology* 10, 103-112

The effects of EPL001 and EPL030 on the attachment of *Pasteuria* to root-knot J2s

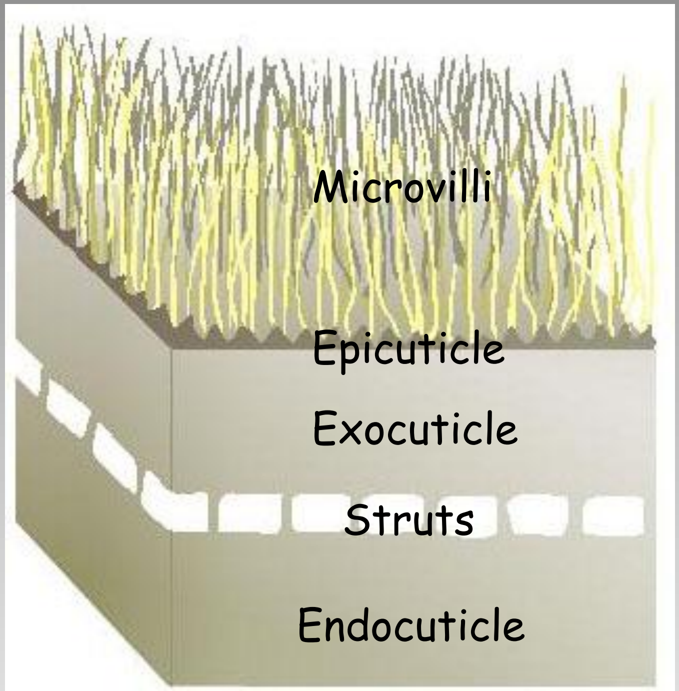
- *Meloidogyne incognita* allowed to hatch in water
- Treat with 1μM of EPL001 and EPL030 (water control)
- At 0, 18, 21 and 27 hrs wash (x3) water
- Endospore (strain RES147) attachment test by centrifugation
- Count endospores adhering to the cuticle

The effects of EPL001 and EPL030 on the attachment of *Pasteuria* to root-knot J2s

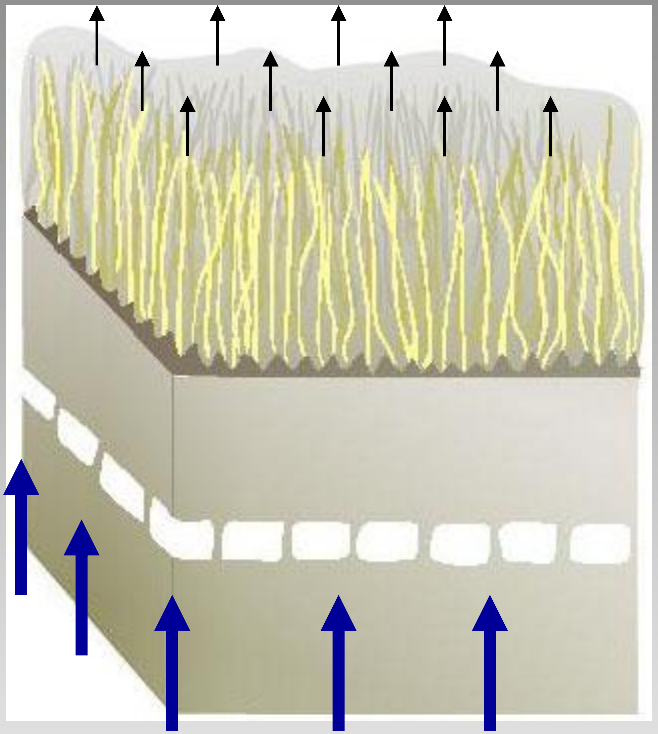


Davies unpublished

Modulation of surface coat through EPL001 and EPL030



Excretory/secretory products



Young — Time line → Old

DAF-2 modulation of E/S products

Davies unpublished

Three types of cuticle variation

- Constituative variation:
  under genetic control and can segregate
- Clonal vartiation:
  probable under epigenetic control
- Induced variation:
  modulated by environmental parameters

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ROTHAMSTED RESEARCH

PASTEURIA BIOSCIENCE
Nematode Control, Naturally

EcoTrain

Plant Nematode Genetics Group
North Carolina State University

bbsrc
biotechnology and biological sciences research council