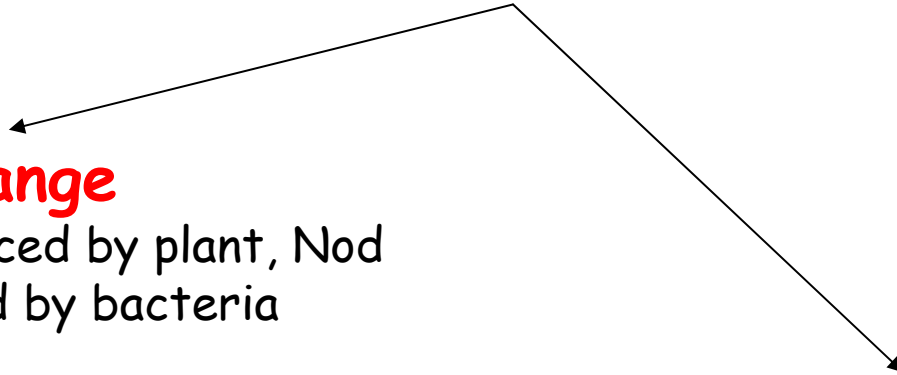


Nutrient and signal exchange between *Photorhabdus* and its invertebrate hosts

Dr David Clarke
University of Bath

Mutualistic interactions

- Beneficial to both organisms
- Often involve:



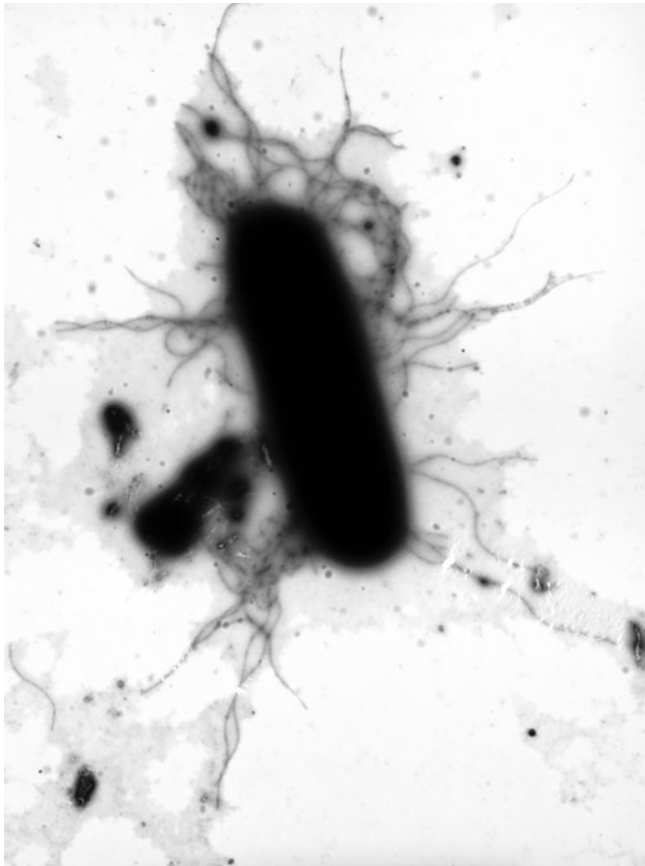
Signal Exchange

Flavanoids produced by plant, Nod factors produced by bacteria

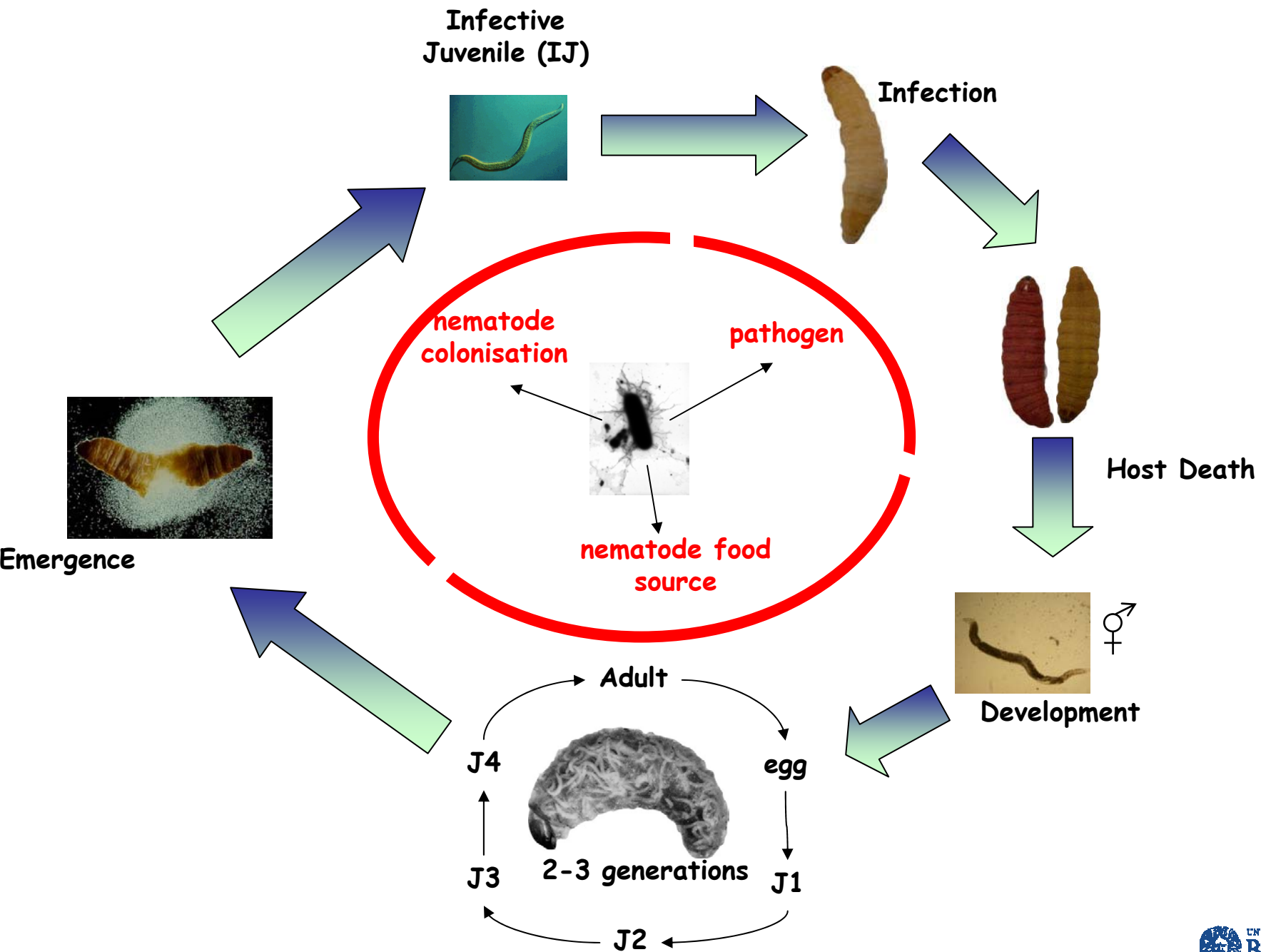
Nutrient Exchange

Plant supplies bacteroid with amino acids; bacteroid supplies plant with ammonia

Photorhabdus



- Gram negative rod
- family *Enterobacteriaceae*
 - close relative of mammalian pathogens
- entomopathogenic bacterium with a complex life cycle





Photobacterium genome

Bioluminescence

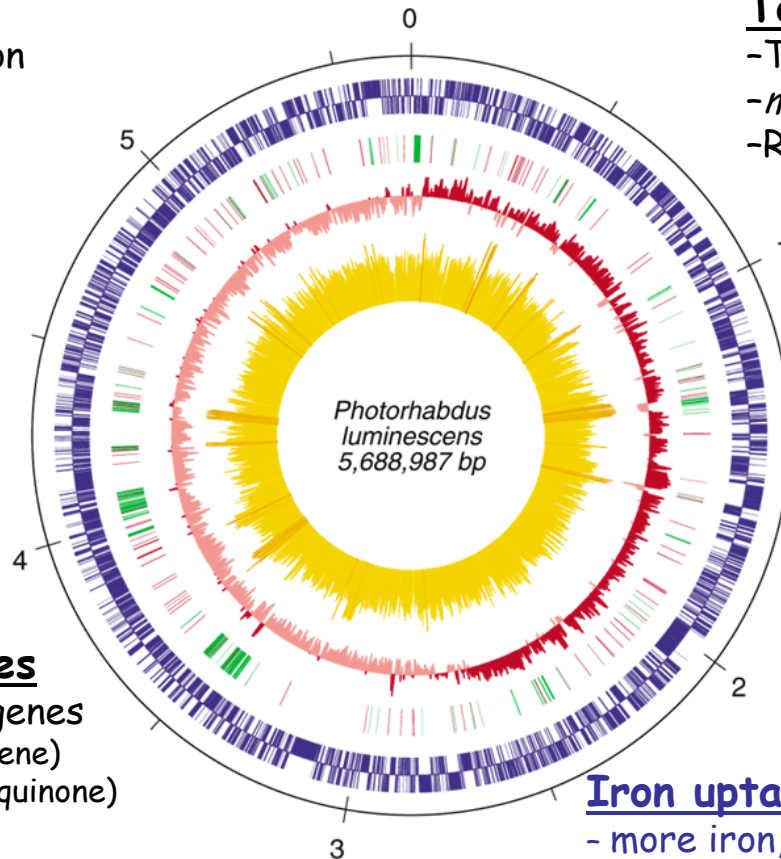
- Product of *luxCDABE* operon

Extracellular enzymes

- lipase (10 genes)
 - phospholipase A and D
- protease
- chitinase

Bioactive molecules

- 33 PKS and NRPS genes
 - Antibiotic (stilbene)
 - Pigment (anthraquinone)
 - Siderophores



Toxins

- Tc toxins
- *mcf*
- RtxA-like toxins

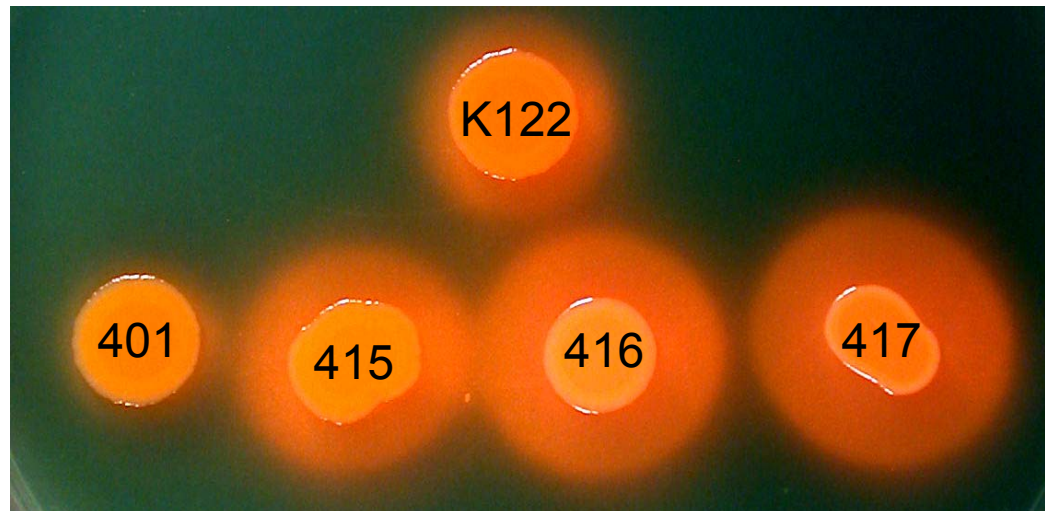
Regulators

- 32 *luxR* genes
- 19 2CP
- 10 σ factors (5 ECF sigma factors)

Iron uptake

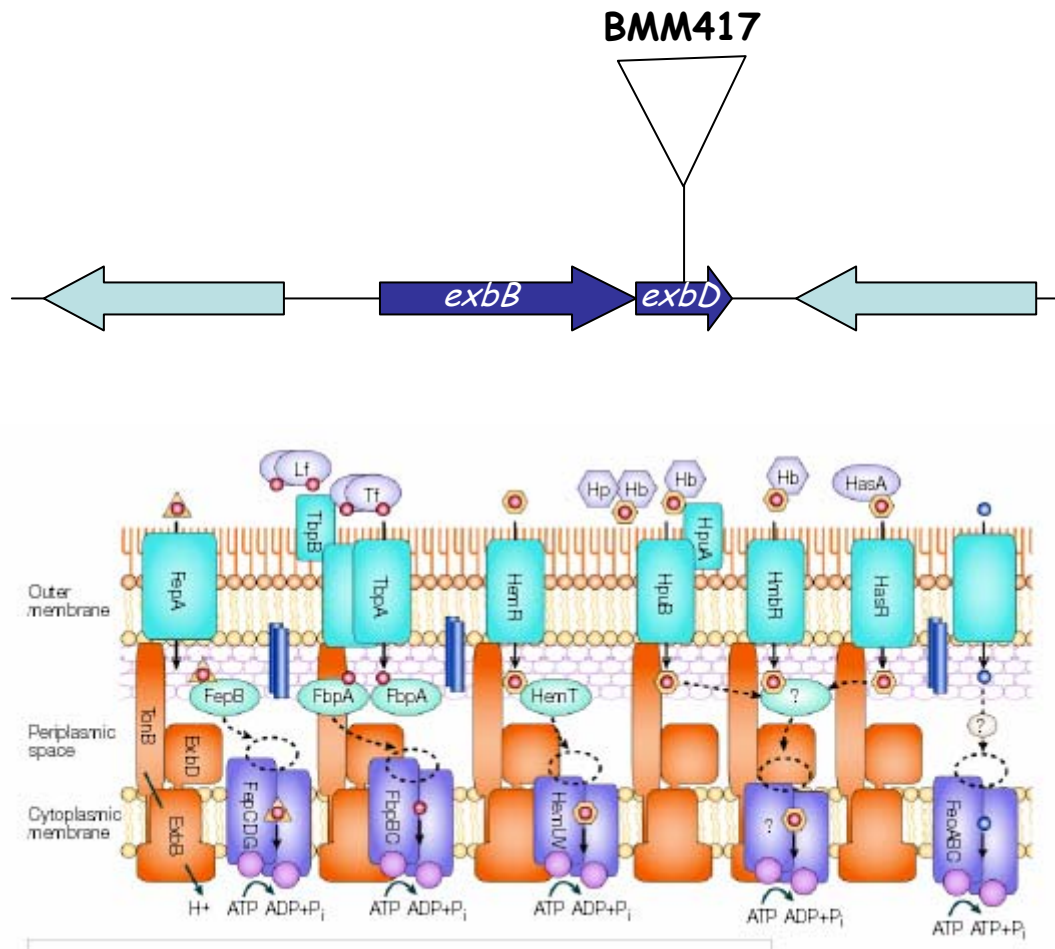
- more iron, heme, hemin and siderophore transporters than any other sequenced bacteria

Siderophore production in *P. temperata* K122



Mutant	CAS phenotype ^a	Gene disrupted	Encoded protein
BMM401	Small halo	<i>phbC</i> ^b	Isochorismate synthase
BMM415	hyper	<i>phbF</i> ^b	Non-ribosomal peptide synthase
BMM416	hyper	<i>phuD</i> ^b	ATP-dependent permease
BMM417	hyper	<i>exbD</i>	ExbD

BMM417 is mutated in *exbD*



Pathogenicity

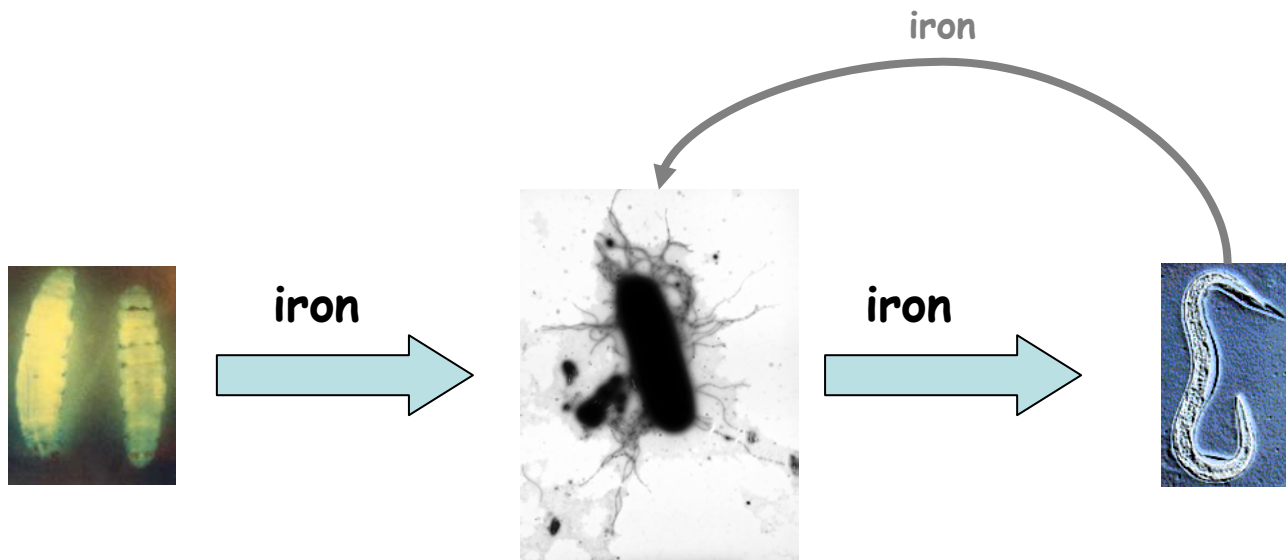
Strain	LT ₅₀ (h) ^a		<i>In vivo</i> doubling time (h) ^b
	- FeCl ₃	+ FeCl ₃	
K122	50.1 ± 2.5 (1.0)	44.3 ± 2.2	1.9 ± 0.3 (1.0)
BMM417	100.7 ± 11.2 (2.0)	46.0 ± 1.0	3.4 ± 0.4 (1.8)
BMM417/pBMM702	56.3 ± 1.0 (1.1)	n.d.	2.2 ± 0.3 (1.1)
BMM401	51.3 ± 2.5 (1.0)	46.3 ± 1.5	n.d.

Nematode growth and development

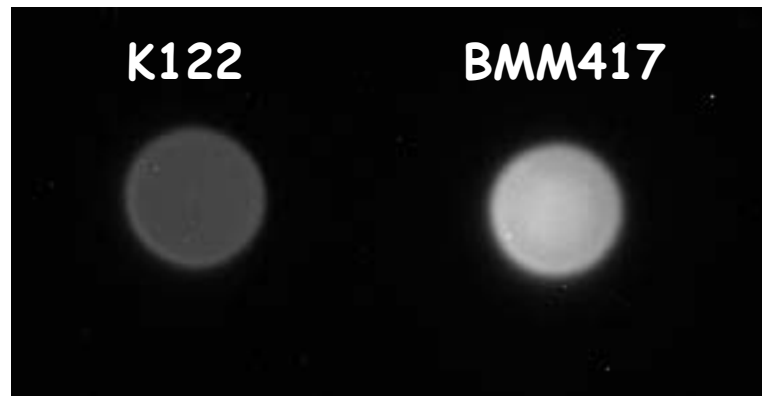
Frequency of nematode growth and development ^a

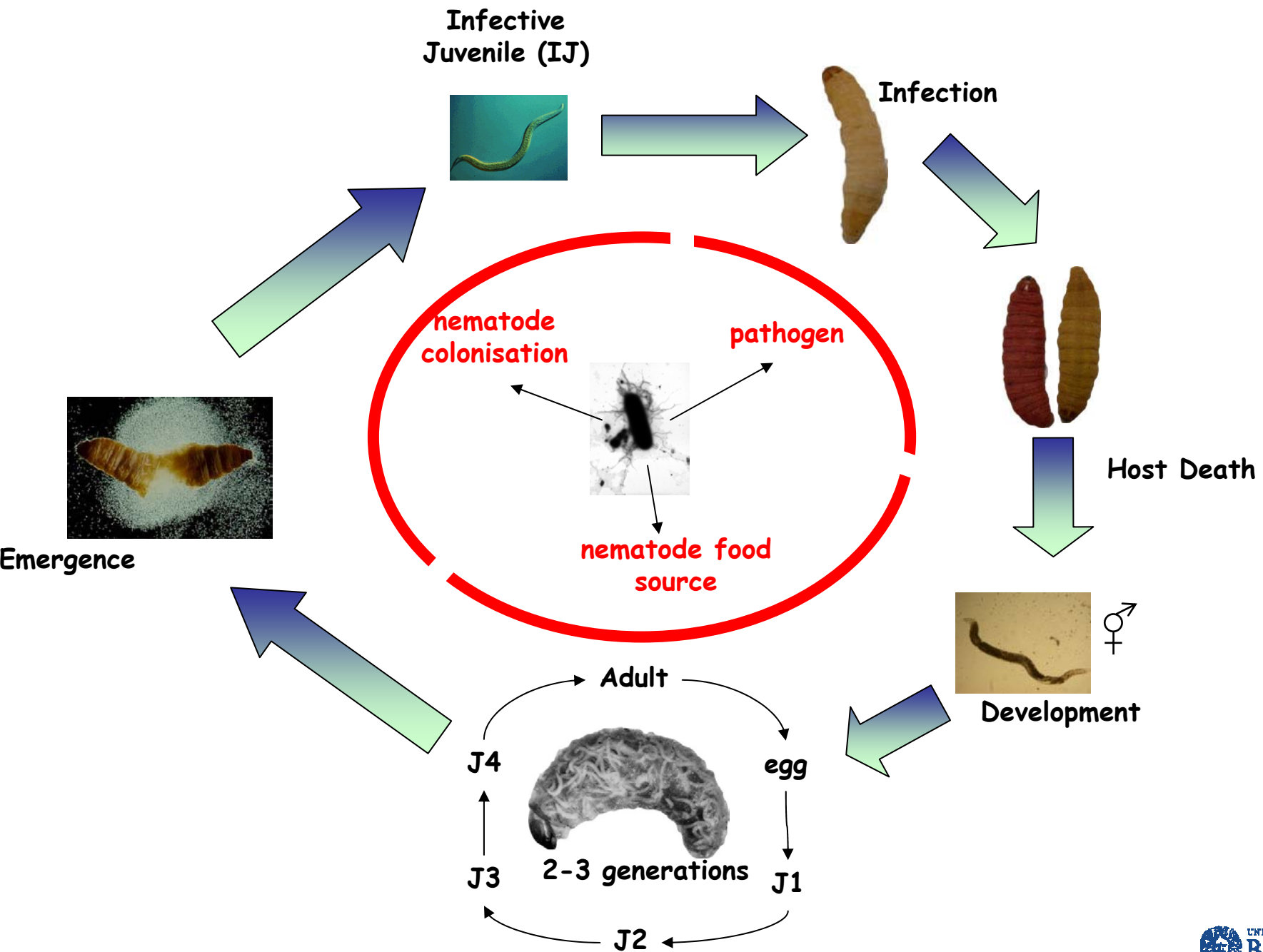
Strain	Frequency of nematode growth and development ^a	
	-FeCl ₃	+FeCl ₃
K122	100%	100%
BMM417	0%	100%
BMM417/pBMM702	90%	90%
BMM401	100%	90%

Nutritional interactions



BMM417 is hyper-bioluminescent





IJ recovery

- Bacterial regurgitation and development of hermaphrodite
- Regurgitation is stimulated by factor(s) in insect hemolymph
- Hermaphrodite development also stimulated by bacterial "food" signals produced in post-exponential phase of growth

Infective juvenile



300-700 μ M

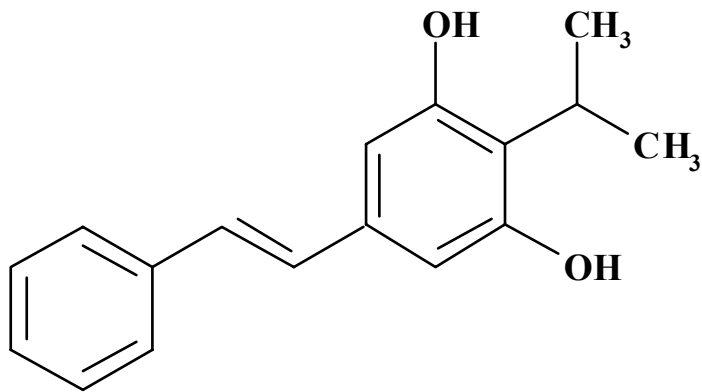
3-4 days

Hermaphrodite

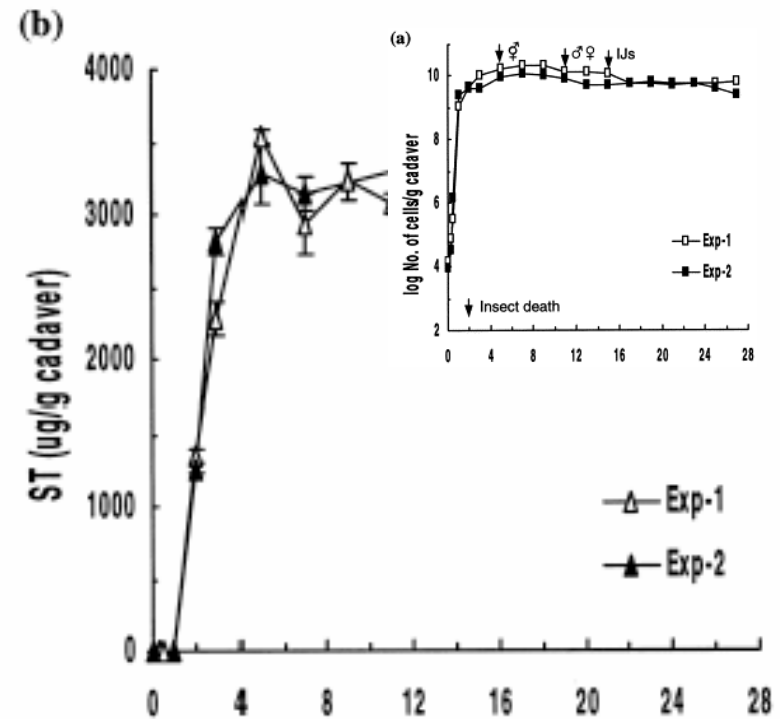


3000-7000 μ M

Photorhabdus produce a stilbene antibiotic

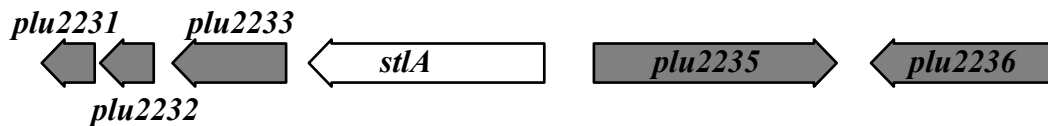
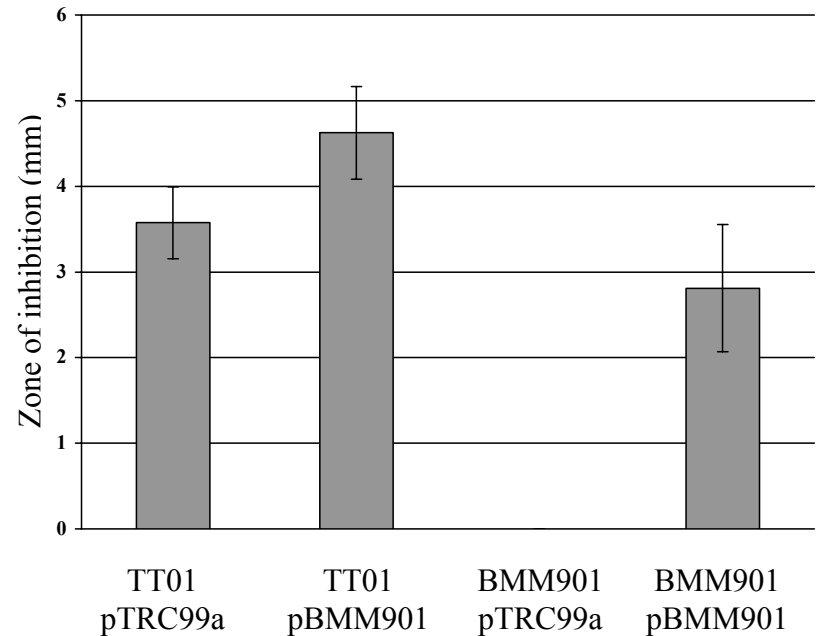
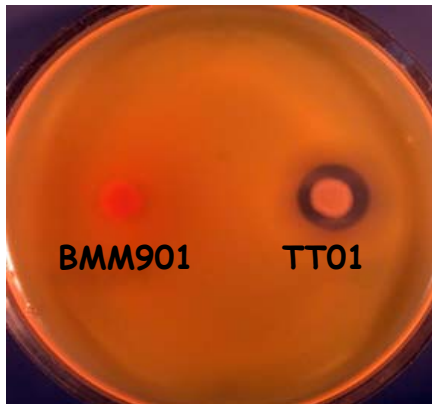


3,5-dihydroxy-4-isopropylstilbene (ST)



Hu and Webster (2000) FEMS Microbiol Lett **189**:219-223.

Identification of *stlA*, a gene involved in ST production



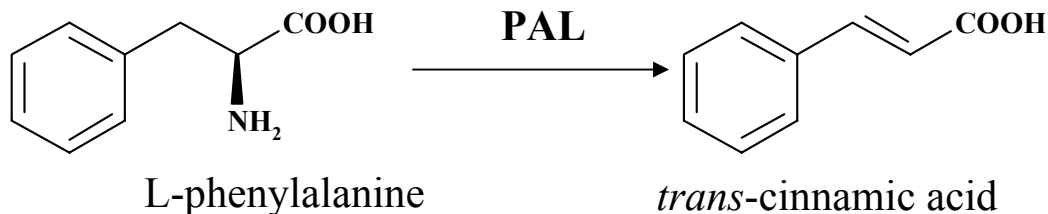
Conserved in *Y. enterocolitica*

StIA is annotated as a histidine ammonia-lyase (HAL)

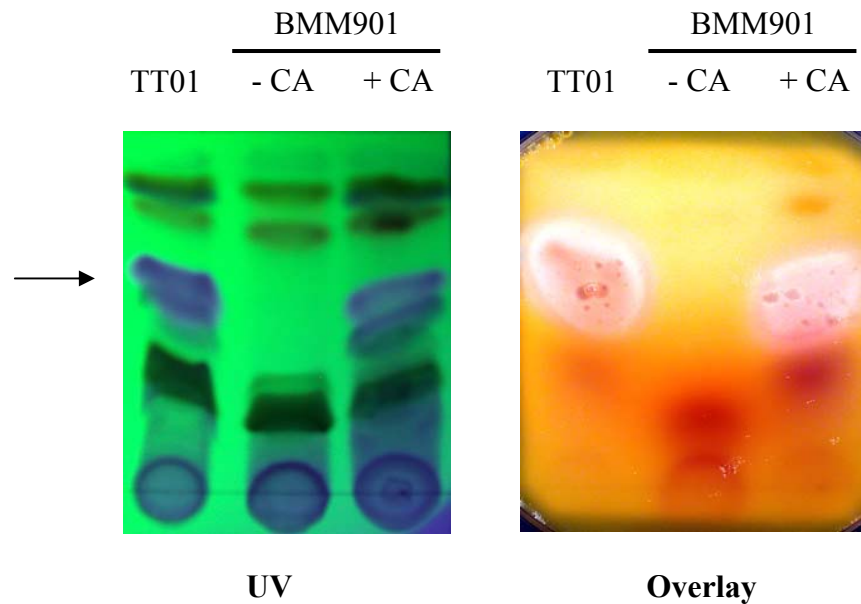
- StIA has 32% identity to HutH, a HAL from *S. enterica*
 - Histidine → urocanic acid
- Using HutH as query can find 2 homologues in TT01 genome; StIA (32% id) and Plu3192 (73% id)
 - *plu3192* encodes the canonical HutH in *P. luminescens* TT01
- What is the activity of StIA?

StlA has some sequence similarity to phenylalanine ammonia-lyase (PAL)

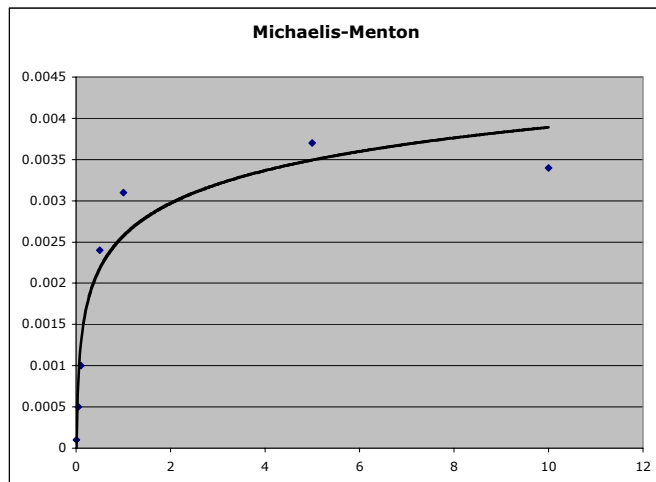
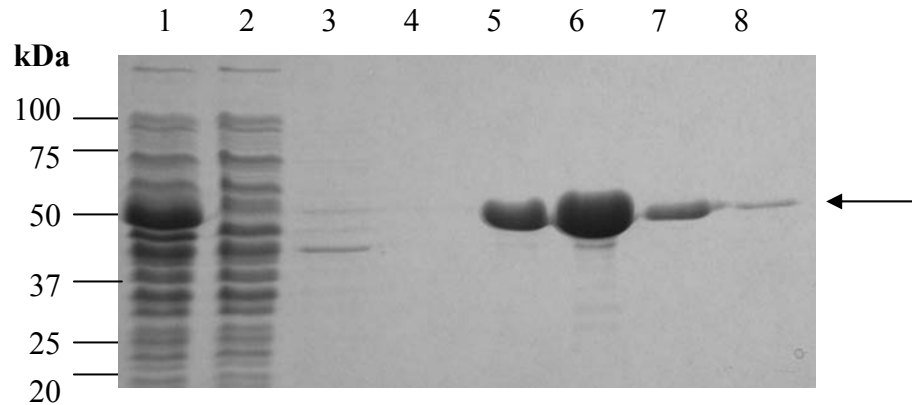
380		412	
G F M I A Q V	N Q E D H	HAL <i>Pp</i> (510 aa)	
G F M I A Q V	N Q E D H	HAL <i>Se</i> (506 aa)	
G F M I A Q V	N Q E D H	HAL <i>Yp</i> (510 aa)	
G F M I A Q V	N Q E D H	HAL <i>Pl</i> (514 aa)	
G F K G A E I	H N Q D V	PAL1 <i>Pc</i> (716 aa)	
G F K G V Q L	Y N Q D I	StlA (532 aa)	



ST production is restored in BMM901 by feeding cinnamic acid

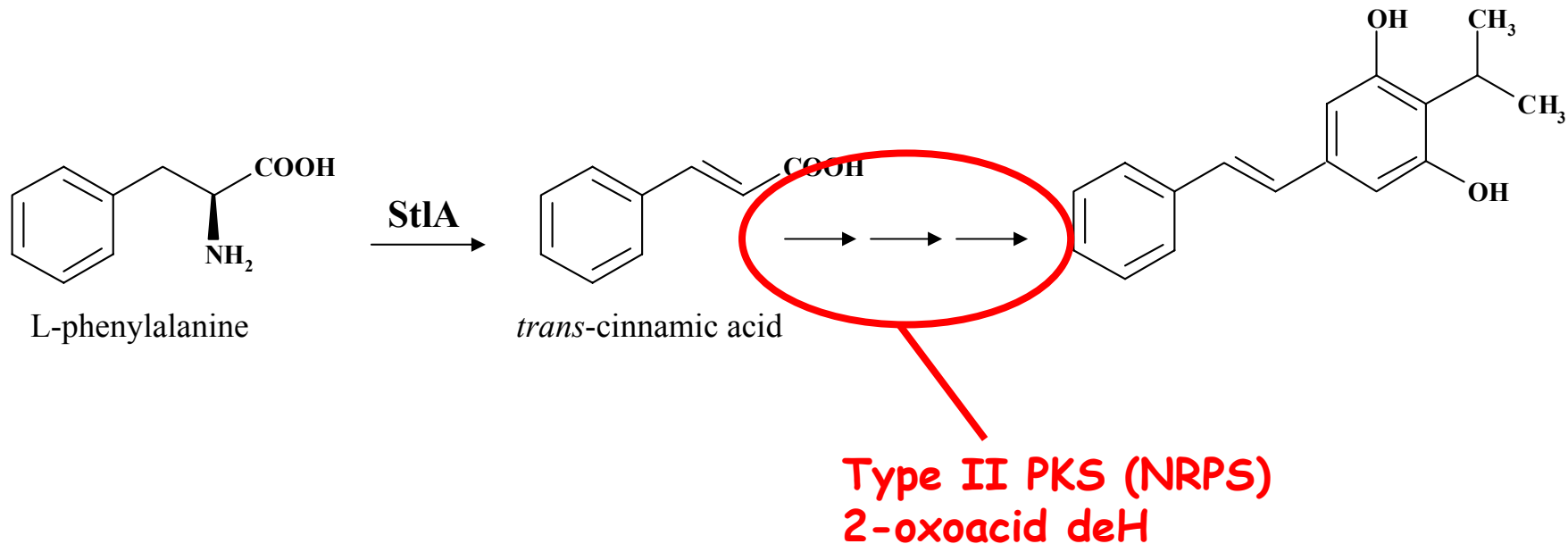


StIA has PAL activity



$$K_m = 320 \pm 35 \mu\text{M}$$
$$U_{\text{max}} = 13.2 \pm 0.02 \text{ pmol s}^{-1} \mu\text{g protein}^{-1}$$

StIA catalyses the first step in ST production



The Molecular Microbiology Laboratory

Photorhabdus

- Dr. Susan Joyce
- Dr. Jane Williams
- Helen Bennett
- Rob Watson
- Hilton McWeeney
- Prof Itamar Glazer

Rcs

- Dr. Lionel Ferrieres
- Ya-hui Huang



The Leverhulme Trust

