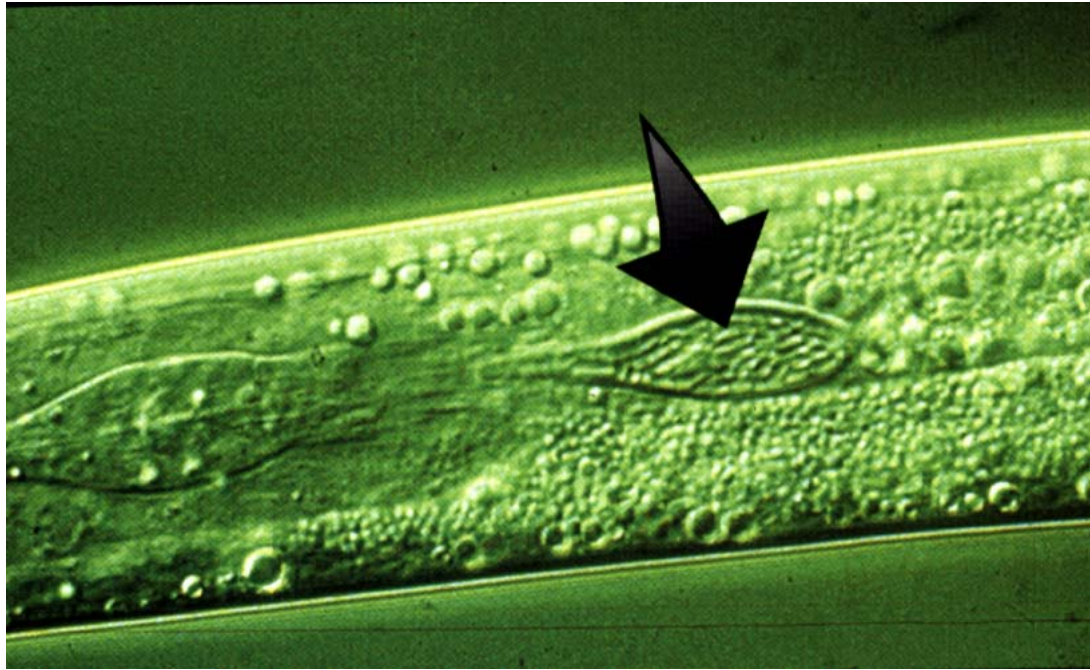


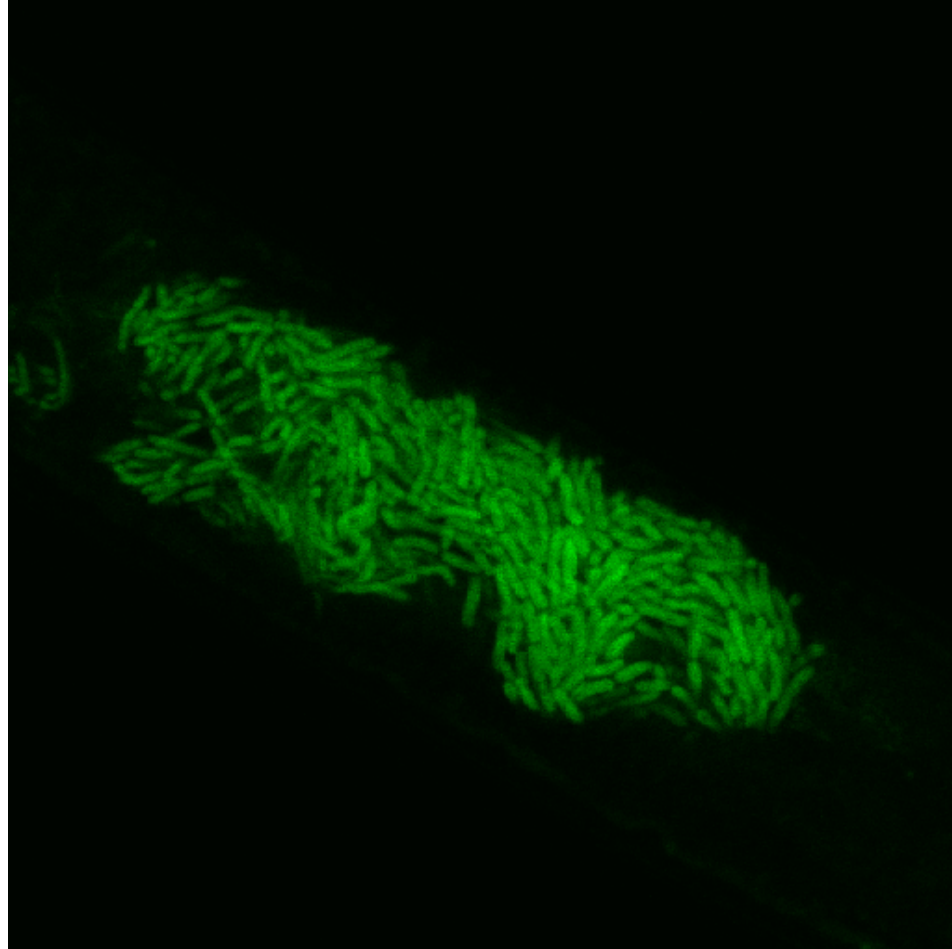
Environmental sensing and  
comparative genomics in  
*Xenorhabdus* spp.

Steven Forst

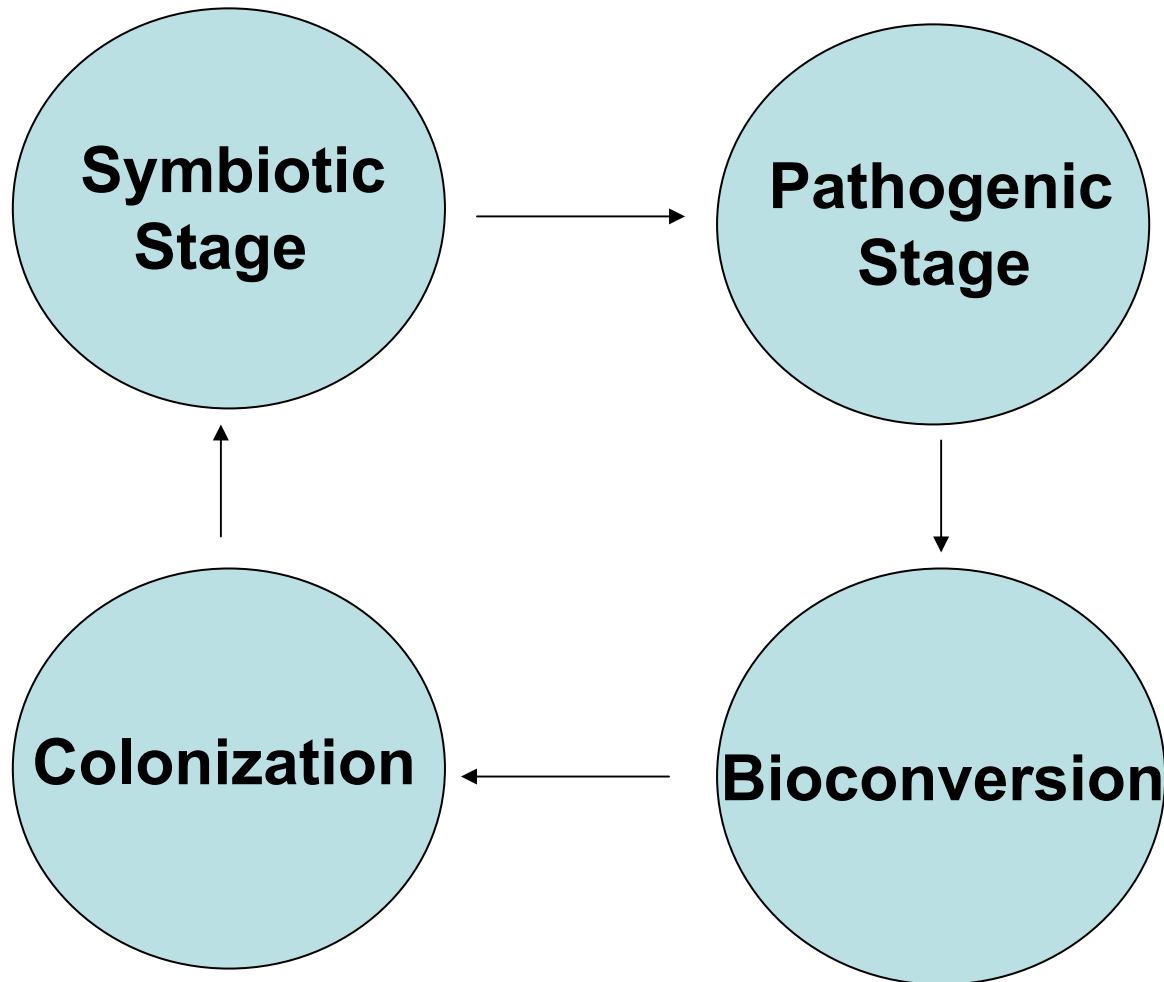
University of Wisconsin-Milwaukee



# GFP-*X.nematophila* in gut vesicle

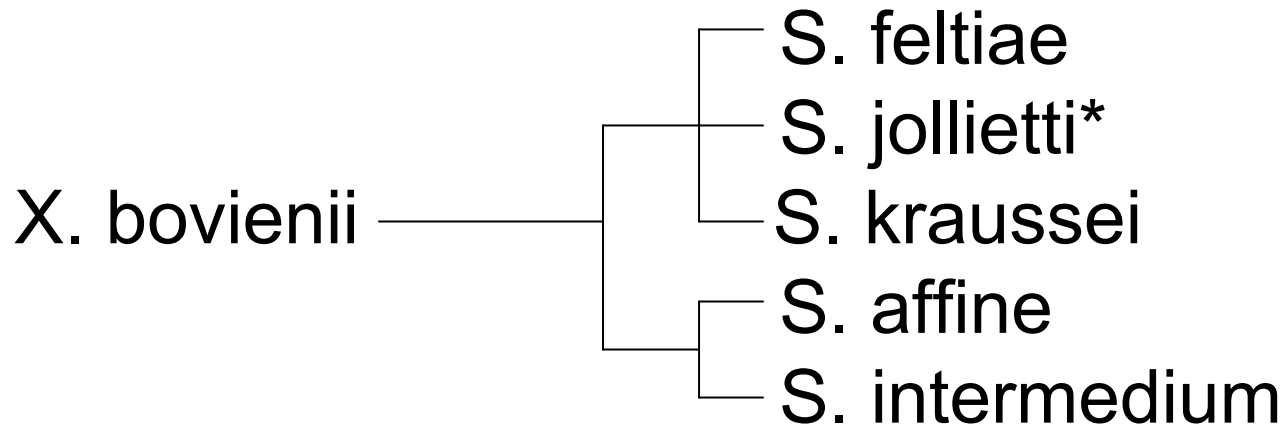


# Life cycle of *X. nematophila*

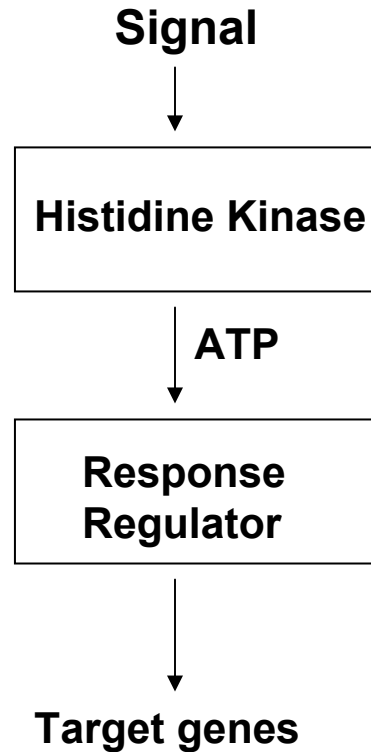


# Species Specificity

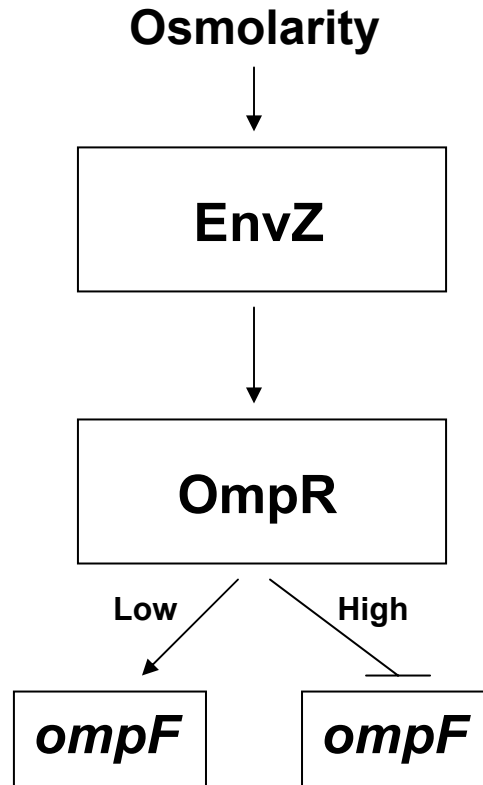
X. nematophila → S. carpocapsae



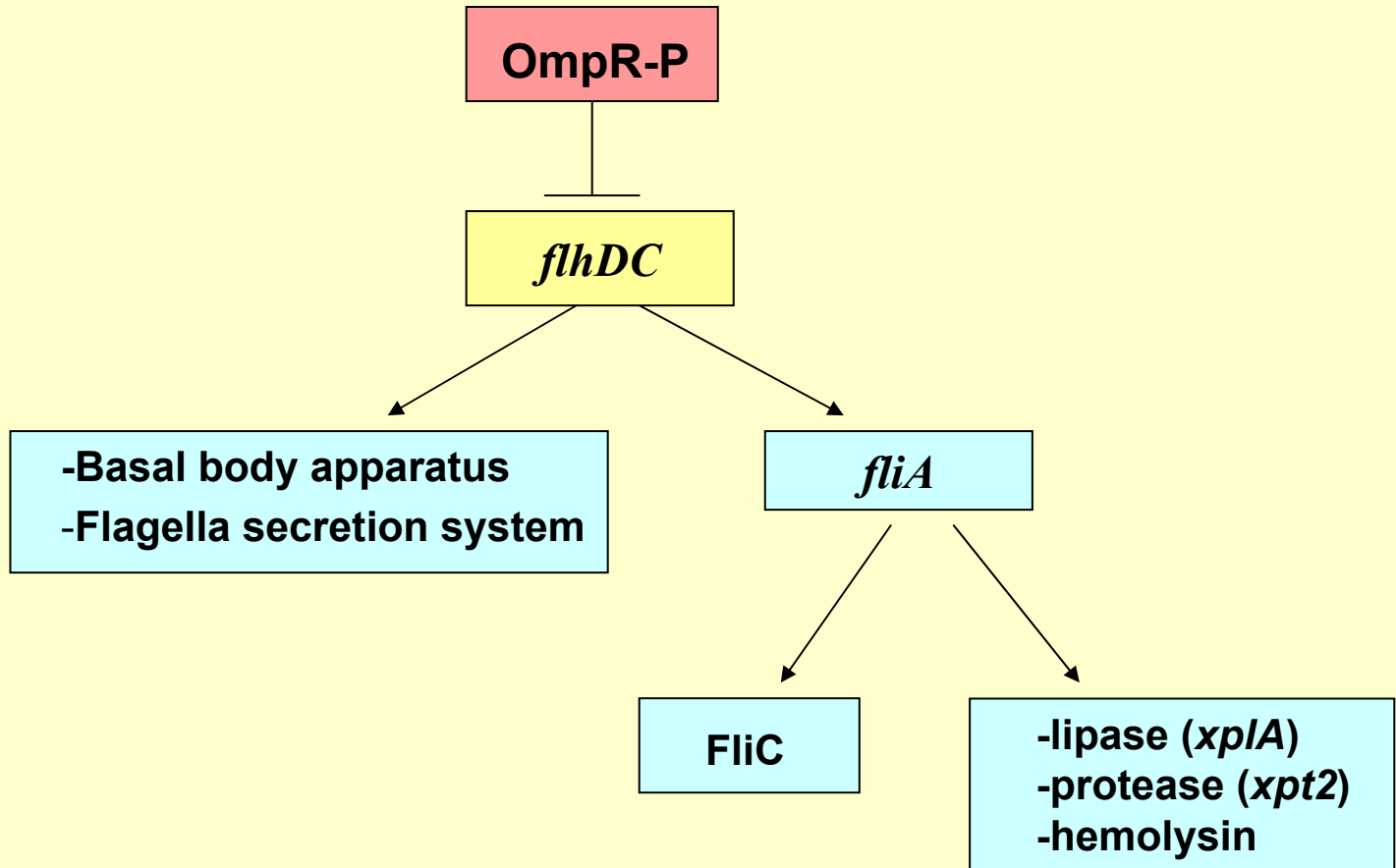
# Two-component signal transduction system



# The EnvZ-OmpR two-component system



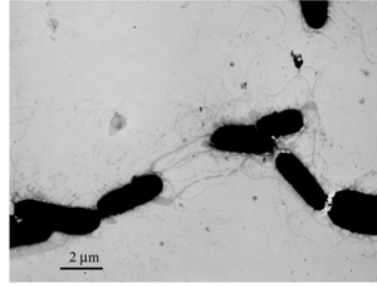
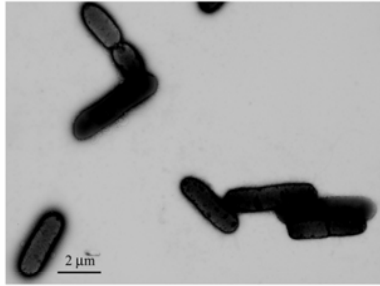
# OmpR regulation of *flhDC* and exoenzyme production



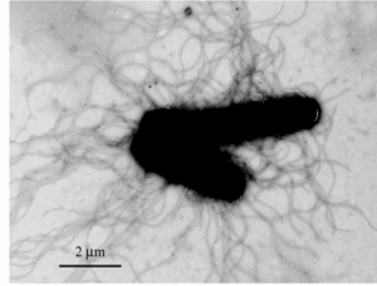
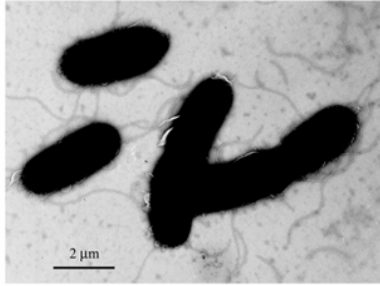
**AN6**

**ABR2**

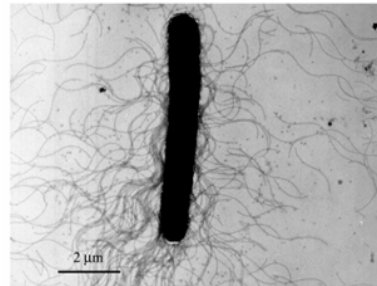
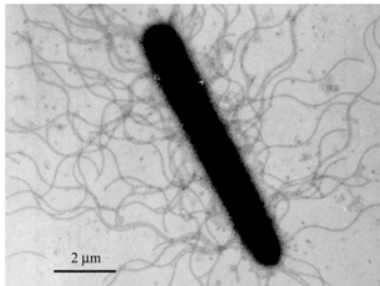
**6 h**



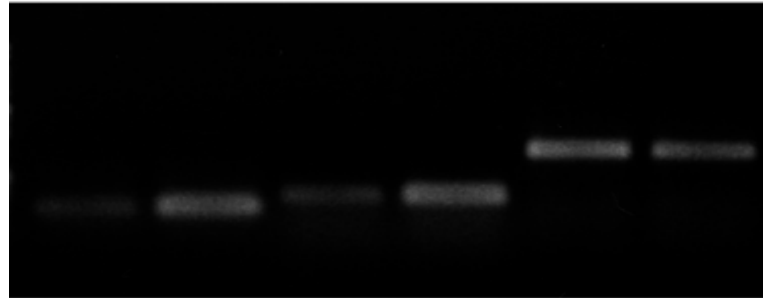
**8.5 h**



**11 h**



**flhDC**                      **fliC**                      **rRNA**  
**1**    **2**                      **3**    **4**                      **5**    **6**



**wt** : lane 1, 3, and 5      *ompR* : lane 2, 4, and 6

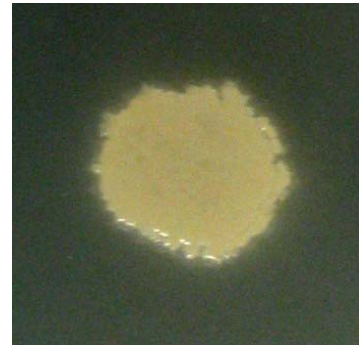
**wt**

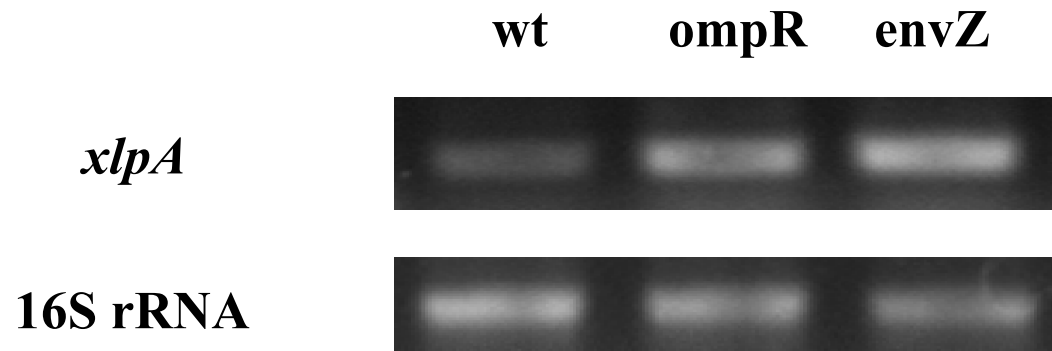
***xlpA***

**Tween 40**



**Tween 60**





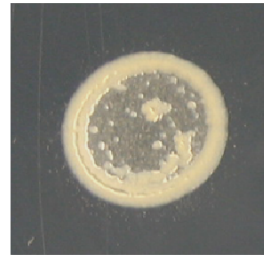
**wt**

**flhC**

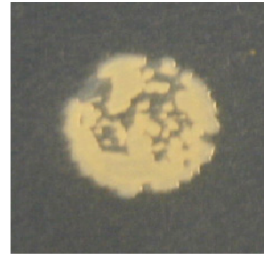
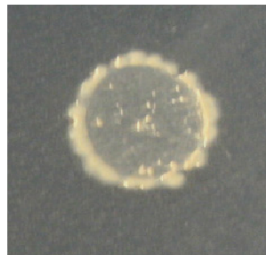
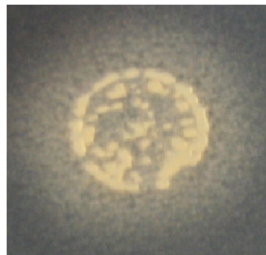
**fliA**

**flhA**

**Tween 40  
lipase**



**Tween 60  
lipase**



*xplA* is regulated by a  $\sigma^{28}$  promoter

	<b>-35</b>	<b>-10</b>
<i>xplA</i>	<b>TAATAAT--N12--</b>	<b>GCCGATA</b>
$\sigma^{28}$ consensus	<b>TAAAGTT--N12--</b>	<b>GCCGATA</b>

**wt**

**flhC**

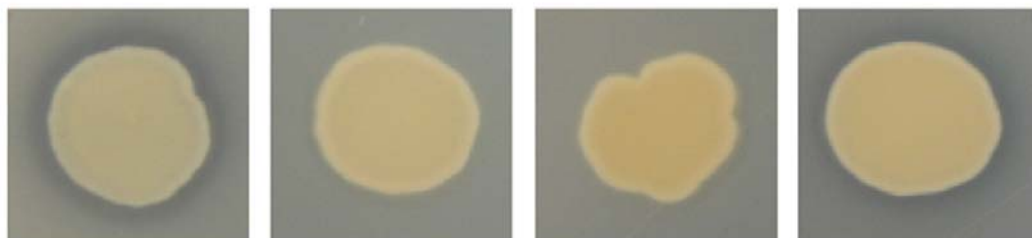
**fliA**

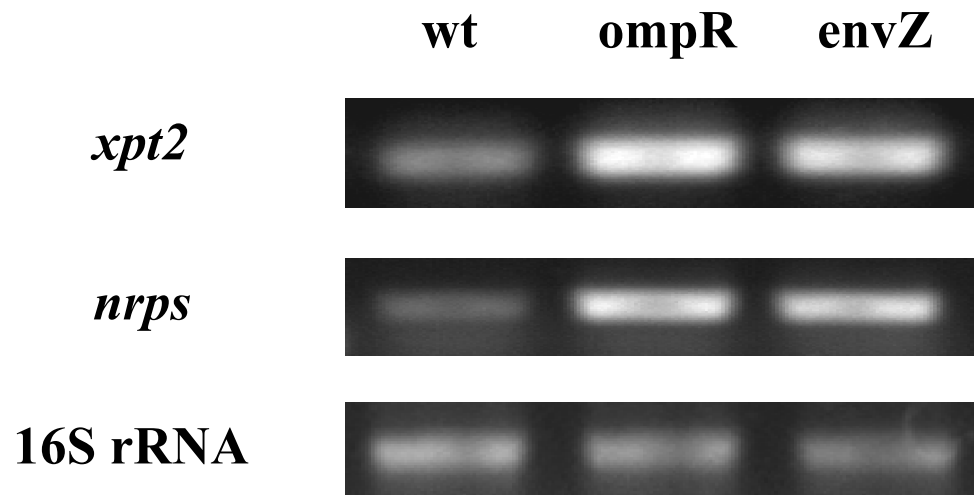
**flhA**

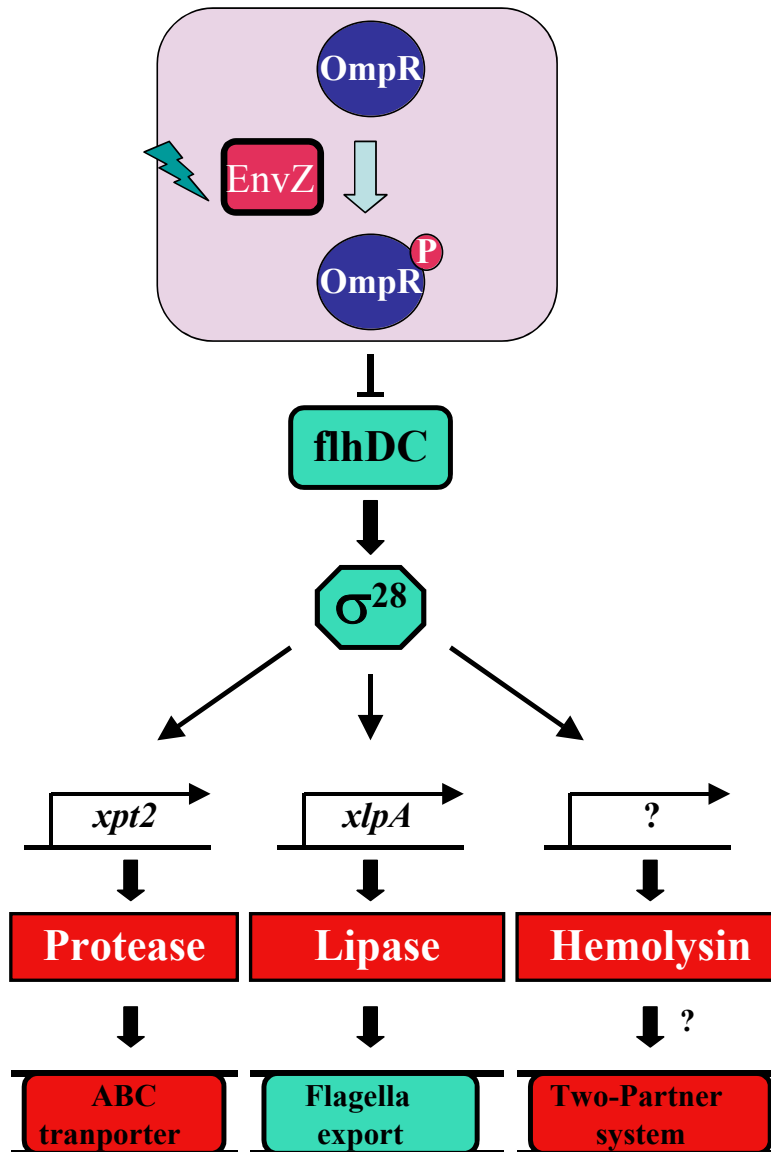
**hemolysin**



**protease**







# Xenorhabdus genome project

- Barry Goldman (Monsanto Co.)
- Heidi Goodrich-Blair (UW-Madison)
- Steven Forst (UW-Milwaukee)
- Creg Darby (U. Alabama)
- Brad Barbezuk (Danforth Plant Science Center)
- Brad Goodner (Hiram College)
  
- USDA (#05502)

# Status of genome project

- *X. bovienii* - 4.3 Mb
  - Assembly completed
- *X. nematophila* - 4.4 Mb + 200 kb plasmid
  - 10 contigs

## COMPARISON OF TWO-COMPONENT SYSTEMS

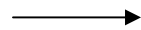
<u>Bacterium</u>	<u>Response regulators</u>	<u>Histidine kinases</u>
<i>S. typhi</i>	34	31
<i>E. coli</i> K-12	33	28
<i>S. flexneri</i>	33	28
<i>E. carotovora</i>	31	25
<i>Y. pseudotuberculosis</i>	27	25
<i>Y. pestis</i>	26	26
<i>P. luminescens</i>	21	18
<i>X. bovienii</i>	19	16
<i>X. nematophila</i>	19	14

## Histidine kinase family

<u>Ortholog</u>	<u>Functions</u>	<u><i>X. nematophila</i></u>	<u><i>X. bovienii</i></u>	<u><i>P. luminescens</i></u>
EnvZ	Porin regulation, virulence	+	+	+
CpxA	Cell envelope stress, protein degradation	+	+	+
KdpD	Potassium transport	+	+	+
PhoR	Phosphate regulation	+	+	+
PhoQ	Magnesium regulation, virulence	+	+	+
NtrB	Nitrogen regulation	+	+	+
UphB	Hexose phosphate uptake	+	+	+
TctE	Citrate transport	+	+	+
CheA	Chemotaxis	+	+	+
RcsC	Capsule synthesis	+	+	+
ArcB	Respiration control	+	+	+
BarA	Hydrogen peroxide sensitivity	+	+	+
YfhK	Unknown	+	+	+
NarQ	Nitrate and nitrite utilization	-	+	+
DcuS	C <sub>4</sub> -dicarboxylic acid utilization	-	-	+
BaeS	Envelope stress response	-	+	+
AstS	Adaptation to stationary phase	-	-	+

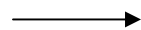
# Quorum sensing systems

**Homoserine  
Lactone (HSL)**



**Intraspecies Signaling**

**Autoinducer 2  
(AI2)**

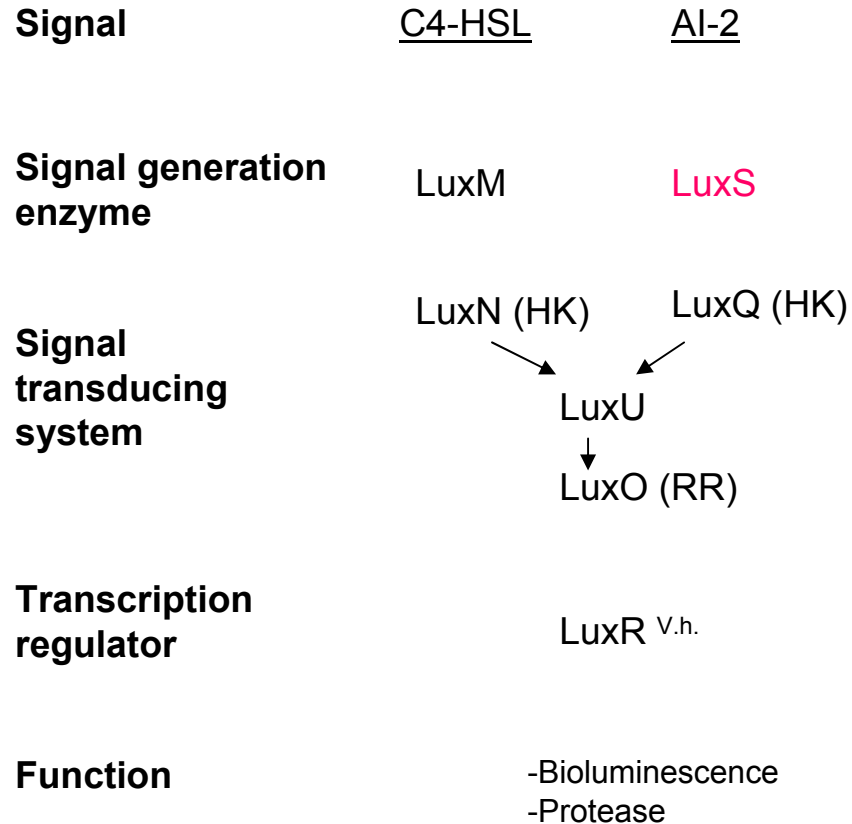


**Interspecies Signaling**

# Quorum sensing systems



# Quorum sensing in *Vibrio harveyi*



# Quorum sensing in *Vibrio fischeri*

Signal	<u>Oxo-C8 HSL</u>	<u>Oxo-C6 HSL</u>
Signal generation enzyme	AinS	LuxI
Signal transducing system	LuxN (HK) ↓ LuxU ↓ LuxO (RR)	
Transcription regulator	LitR	LuxR <sup>V.f.</sup>
Function	-Bioluminescence -Initiation of symbiosis	-Bioluminescence -Maintenance of symbiosis

# Quorum sensing in *Salmonella typhimurium*

<b>Signal</b>	Exogenous <u>HSL</u>	<u>AI-2</u>
<b>Signal generation enzyme</b>		LuxS
<b>Transcription regulator</b>	SdiA	LsrR
<b>Function</b>	-Resistance to Complement	-Regulate <i>lsr</i> operon Uptake of AI2

## Summary of quorum sensing systems

	<u>X. nematophila</u>	<u>X. bovienii</u>	<u>P. luminescens</u>	<u>Y. pestis</u>
<b>LuxS</b>	<b>yes</b>	<b>yes</b>	<b>yes</b>	<b>yes</b>
<b>LuxI/R</b>	<b>no</b>	<b>no</b>	<b>no</b>	<b>2 copies</b>
<b>SdiA</b>	<b>no</b>	<b>no</b>	<b>yes</b>	<b>yes</b>
<b>LsrR</b>	<b>no</b>	<b>no</b>	<b>yes</b>	<b>yes</b>

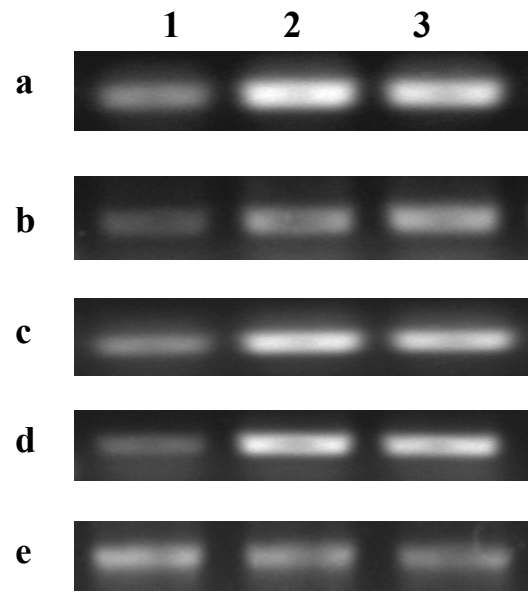
Organism	Number of Porins
<i>E. coli K12</i>	7
<i>Yersinia pestis</i>	5
<i>Photorhabdus luminescens</i>	3
<i>Xenorhabdus bovienii</i>	3
<i>Xenorhabdus nematophila</i>	3

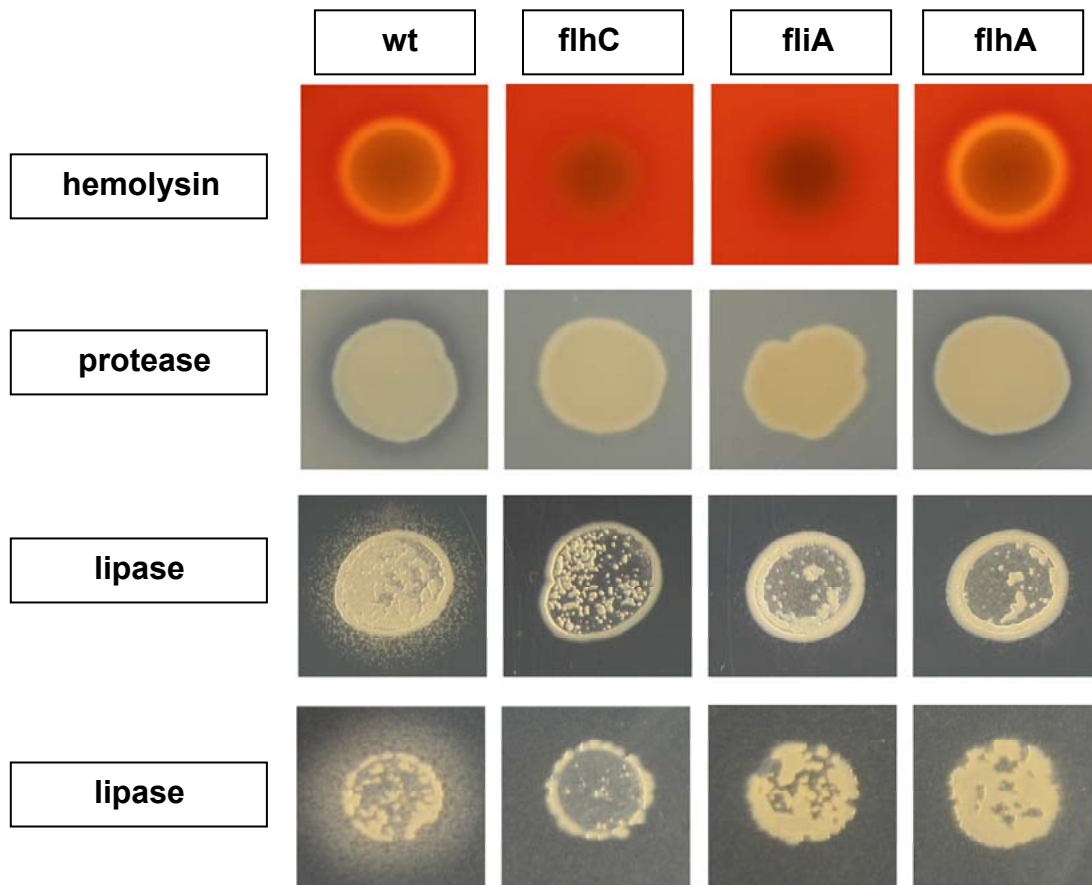
# Summary

- The number of 2CS is significantly reduced in *Xenorhabdus*
- *Xenorhabdus* does not possess unique 2CS
- EnvZ-OmpR regulates motility and exoenzyme production
- *luxS* is only quorum sensing gene so far identified
- *Xenorhabdus* lacks LuxI/R, LsrR and *lsr* operon, and SdiA regulator
- Overall reduction in potential to sense and adapt to diverse environmental conditions

# Acknowledgements

- Dongjin Kim
- Ransome van der Hoeven
- Holly Snyder
- Brian Boylan
- Nick George





# Environmental Adaptation

## Nematode gut vesicle

- monoclonal colonization
- outgrowth in vesicle
- persistence

## Insect hemolymph

- intracellular pathogen?
- survive and grow in nodule
- high cell density in hemolymph

**OmpR**

***flhDC***

**Class II genes  
(*fliA*)**

- **Lipase (*xplA*)**
- **Protease (*xpt2*)**
- **Hemolysin**
- **Nonribosomal peptide synthase**
- **Others?**