Quorum sensing in plant-associated bacteria

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Outline

• What is quorum sensing?
• QS in plant associated bacteria
  – What traits are regulated by QS?
  – What benefits does QS-regulation provide?
• Response to QS
  – In plants
  – In other bacteria
Luminescence in *Vibrio fischeri*

*Vibrio fischeri*  
*Euprymna scolopes*
Luminescence is induced at high cell densities according to Hastings and colleagues.

Graph showing the relationship between luminescence activity and time, with an increase in activity over time at high cell densities.
Spent supernatant from dense cultures induces early luminescence production

The “activator” in spent supernatant:

\[ N-(3-\text{Oxohexanoyl})-\text{L-homoserine lactone} \]
The mechanism of signaling in *V. fischeri*

- low cell population densities
- diffuse environment (seawater)
The mechanism of signaling in *V. fischeri*

- *luxI* synthase
- *luxR* activator

- high cell population densities
- confined environment (squid)

Induce *lux* gene expression (and others)

= [AI molecule diagram]
Why is light production linked to cell density?

Surface seawater
Blue = DAPI stained bacteria
Green = Added microspheres for size comparison (5 μm)

GFP-tagged *V. fischeri* colonizing squid light organ
(Nyholm *et al.*, 2000. *PNAS*)

(Smith *et al.*, 2000. ODP Technical Note)
Moonlight

Shadow

Counterillumination
Quorum sensing:

“The term "quorum sensing" describes the ability of a microorganism to perceive and respond to microbial population density, usually relying on the production and subsequent response to diffusible signal molecules.”

LuxI-type synthases produce a variety of fatty acyl-HSL signals

>300?? species use fatty acyl-HSL signaling

~30 different signal structures described

New diversity: first aryl-HSL found in *R. palustris*
Other kinds of signals:

QS in plant-associated bacteria

• How might QS benefit a plant pathogen, commensal or mutualist?

  – What traits would benefit a population but not an individual cell?

  – Does the specific plant-associated niche make a difference (i.e. leaf surface vs. rhizosphere vs. intracellular)?
Virulence factors: “sneak attack” by *Erwinia carotovora*

PCWDE = plant cell wall degrading enzymes  
PDR = plant defense response

Mäe *et al.*, 2001. *MPMI.*
Evidence for sneak attack:

Mäe et al., 2001. MPMI.
Testing “sneak attack” with transgenic tobacco

Plant expressing *expI*, making C8-HSL

Inoculated with $2 \times 10^6$ CFU *Erwinia carotovora*

Vector control plant

Mäe *et al.*, 2001. *MPMI.*
QS-regulated antimicrobial production

Violacein in *Chromobacterium violaceum*

Phenazine antibiotics of *Pseudomonas chlororaphis*

Bactobolin of *Burkholderia thailandensis*

Carbapenem of *Erwinia carotovora*
Hypotheses to explain benefit of QS-regulation of antimicrobials:

- Minimizing cost/benefit ratio:
Does exposure to a low dose of a QS-regulated antimicrobial induce increased tolerance in a sensitive species?
Rhamnolipids of *Psuedomonas aeruginosa* kill *Bacillus subtilis*

- Both species are saprophytes, can colonize plant rhizosphere.

*P. aeruginosa* rhamnolipids


An *et al.*, submitted.
Does *B. subtilis* respond to a subinhibitory dose of rhamnolipids?

- *B. subtilis* + subinhibitory dose of rhamnolipids
- *B. subtilis* + no rhamnolipids

1. Incubate in PBS
2. Resuspend in fresh buffer, add inhibitory dose of rhamnolipids
3. Measure *B. subtilis* survival over time
B. subtilis adapts in response to a low dose of rhamnolipids

An et al., submitted.
Hypotheses to explain benefit of QS-regulation of antimicrobials:

• Defense of newly liberated resources:
QS-regulation to coordinate disease progression

Sweet corn - Susceptible varieties may be systemically infected as late as the 9-leaf stage. Field corn - Few plants are infected systemically due to adequate levels of host-resistance. Most infections are local, leaf blight symptoms.

**LEAF BLIGHT PHASE**
Bacteria are spread by multiple generations of corn flea beetles during the growing season.

When plants are susceptible, leaf lesions are long. When plants are resistant, leaf lesions are short.

**SEEDLING WILT PHASE**
Early infection of susceptible varieties may lead to wilting, stunting and sometimes death.

Flea beetles that overwinter feed on corn seedlings and transmit *Erwinia stewartii* to feeding wounds through feces. Flea beetles emerge in early spring when crops are planted.

Systemic infection from the seedling wilt phase can result in infection of kernels in susceptible varieties.

Inoculum overwinters in association with adult corn flea beetles. Because seed-borne transmission rates are extremely low, seedborne inoculum is insignificant in the epidemiology of Stewart’s wilt in areas where the flea beetles occur.

Drawing courtesy Vickie Brewster.
QS regulation of extracellular polysaccharides- *Pantoea stewartii*

von Bodman *et al.*
1998 *PNAS*
Regulation of EPS in *P. stewartii*

QS and EPS affect attachment of *P. stewartii*

![Graph showing attachment A570 for different conditions: WT, ΔesaR, ΔesalR, Δesal, and EPS gene mutants. The graph indicates a significant increase in attachment for Δesal compared to WT and other mutants.]
**P. stewartii** QS mutants *in planta*

- Reduced dissemination:

![Graph showing distance vs. genotype](image)

Koutsoudis *et al.* 2006 *PNAS*
*P. stewartii* xylem colonization
Responses to QS-plant and bacterial

• Can plants detect and respond to acyl-HSLs?

• Do plants manipulate QS?

• Can non QS-bacteria manipulate or interfere with signaling by their neighbors?
A model plant to measure response to acyl-HSLs: *Medicago truncatula*

- Legume, close relative of alfalfa
- Forms symbiosis with N-fixing, QS-bacterium *Sinorhizobium meliloti*
- Roots readily colonized by saprophytes with QS
Acyl-HSL addition to *Medicago truncatula*

- Accumulation of 150 proteins change by proteomics

- ~66% of changes were dependent on particular acyl-HSL signals (C16- vs. 3oxoC12-HSL)

- May influence auxin balance- auxin responsive elements are increased

![Control and acyl-HSL treated samples with GH3-GUS expression](image.png)
Extracts of *M. truncatula* seedlings contain QS signal mimics

Gao et al. 2003 MPMI
“Quorum quenching” = signal degradation
Bacillus cereus and quorum quenching

- Organisms from *B. cereus* group degrade acyl-HSLs via lactonase activity (encoded by *aiiA*).

- *B. cereus* and antibiotic producers commonly inhabit the same environments (soil, plant roots).
Does the acyl-HSL lactonase of *B. cereus* influence its competitiveness?
B. cereus lactonase mutant in competition with C. violaceum

C. violaceum wt  C. violaceum QS-

+ B. cereus wt
B. cereus lactonase mutant in competition with C. violaceum
**B. cereus** lactonase mutant in competition with **C. violaceum**

Starting CFUs of **B. cereus** and **C. violaceum**.