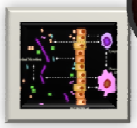
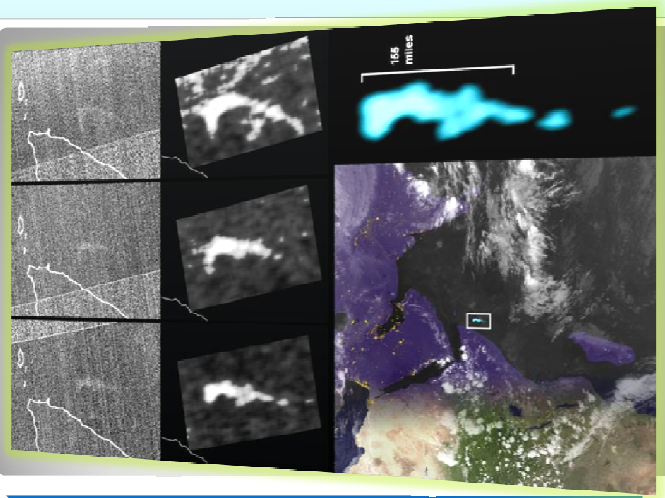
Listening In As Bacteria Talk"

Nuvee Prapasarakul
Seminar in Vet Microbiology
23 February 2009



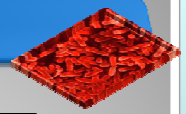
"I THINK THAT A MULTIPLE OF BACTERIA ARE STRONGER THAN A FEW AND THUS UNION ARE ABLE TO OVERCOME OBSTACLES TOO GREAT FOR THE FEW"

- (Smith, 1905)



Steven D. Miller, Naval Research Laboratory (NRL) and Steven H. D. Haddock, Monterey Bay Aquarium Research Institute; Christopher D. Elvidge, National Geophysical Data Center; and Thomas F. Lee, NRL)

Seminar Topic Tresor'



- Aims
- Quorum Methods
- Types
- Impacts
- Protections and Controls
- Question?

AIMS of Communication


Bioluminescenc e


Virulence

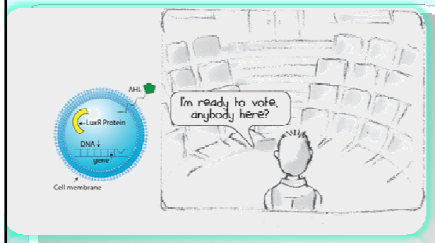

Antibiotic production


Biofilm formation


Symbiosis

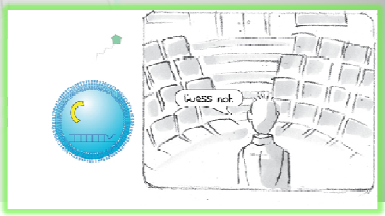
Biofilms: *The good, the bad, and the slimy*??

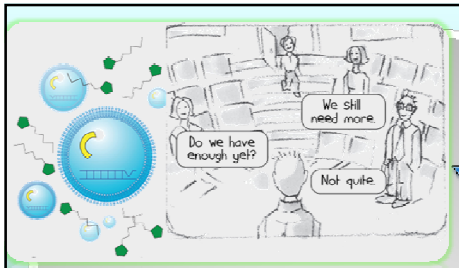
http://www.whoi.edu.



1. Bacteria constantly produce molecules called acylated homoserine lactones (AHLs) and release them into the environment.

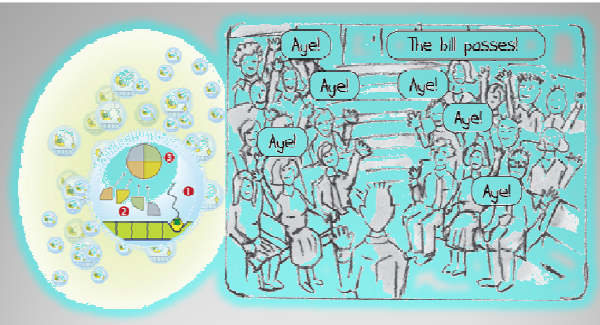
2. If other bacteria are not in the vicinity, the AHLs will soon degrade and bacterial "silence" will prevail.





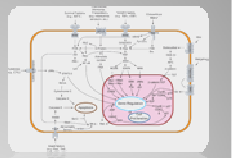
3. But if enough AHL-producing bacteria are nearby, the concentration of AHLs outside the bacteria will rise, signaling the increase in cell density around them.

4. When a quorum of bacterial cells is reached, AHLs are able to bind to receptor proteins called LuxR. (2) That changes LuxR's shape so that it can bind to DNA in the bacteria, like a key in a lock.

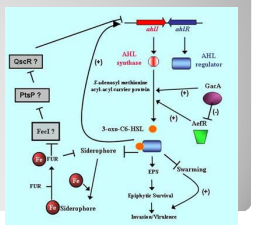


5. The LuxR "key" turns on a string of genes (1) that produce the components (2) to make an enzyme called luciferase (3), which generates light (bioluminescence). By similar biochemical processes, bacteria can produce several other collective behaviors.

Chemical signaling in Bacteria



- Oligopeptide: Gram positive bacteria
  Staphylococcus sp.
- AHL (N Acyle Homoserine Lactone): Gram negative bacteria
  Pseudomonas areuginosa
- AI2 (LuxS/ Autoinducer 2): Gram positve and gram negative.
  Salmonella Typhimurium and *V. harveyi*



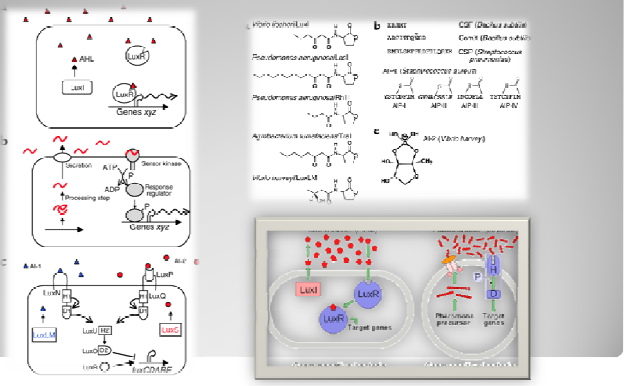
Metabolic costs of signal production

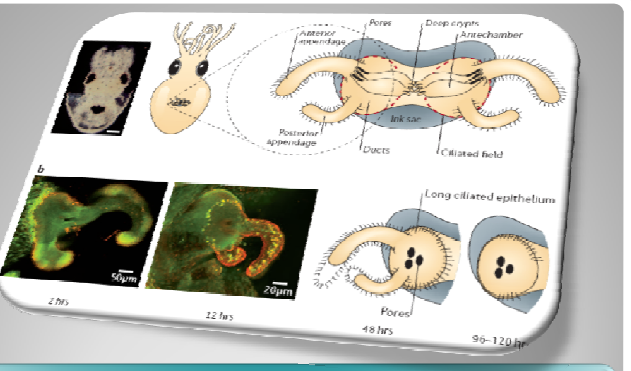
Signal	Metabolic Cost*	Example
Oligopeptides	High	184 ATP (for the AgrD protein of *Staphylococcus aureus*, 46-amino-acid preprotein)†
N-acyl homoserine lactones	Intermediate	8 ATP (for butyryl-homoserine lactone, C4-HSL, produced by RhlI in *Pseudomonas aeruginosa*)§
Autoinducer-2	Low/none	0–1 ATP

Quorum sensing structure

- Organism — Living
- Gene and regulatory protein = — Technology tools
- Autoinducer — Media and signal
- Membrane bound — Antenna or connecting box
- Regulatory system — Censer department (n.u.a.)
- AI2: — The other signals (eg AIS or DTAC)

Three canonical quorum-sensing circuits in bacteria and Structures of different bacterial autoinducers.





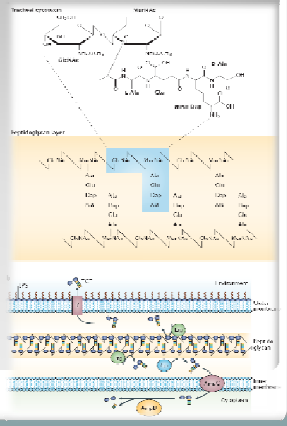
Peptidoglycan and *Euprymna scolopes- Vibrio fischeri* symbiosis. (Cloud-Hansen et al., 2006 in Nature review)

Peptidoglycan and microbial interactions

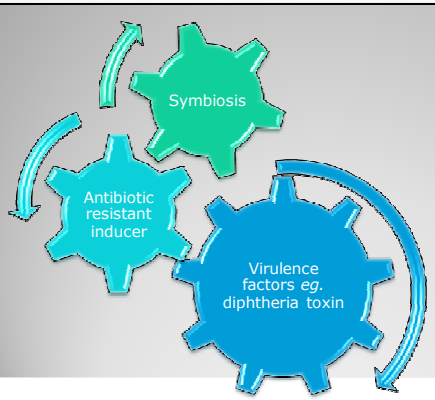
- PG: structure
  - Glycan
    - *N*-acetylmuramic acid
    - *N*-acetylglucosamic acid
  - Peptide
    - l-alanine
    - d-glutamic acid
    - *meso*-diaminopimelic acid

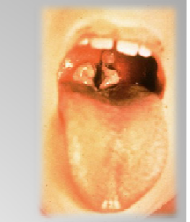
PG-polymers causes damage to ciliated cells

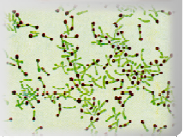
N. gonorr hoeae H. pylori H. influenzae S. flexneri



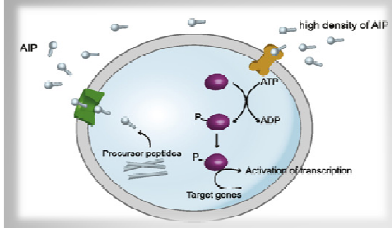
PG acts as autoinducer

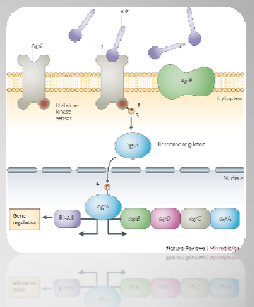






Two component signal transduction in Gram positive bacteria

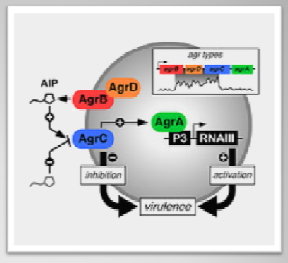




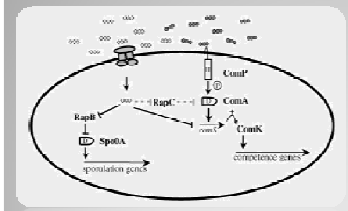
2 component type membrane bound sensor histidine kinase

Quorum sensing control of competence and sporulation in *Staphylococcus aureus*.

- Small number
  attachment and colonization
- Large number
  Release toxins



Quorum sensing control of competence and sporulation in *Bacillus subtilis.*



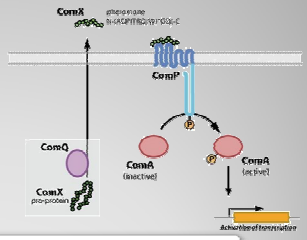
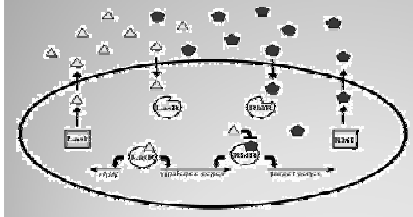
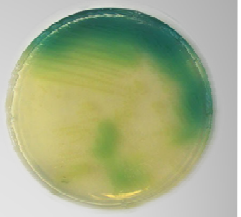


Table 1. Genes involved in Gram positive quorum-sensing and function of protein.

comX	Decapeptide pheromone, secreted.
comQ	Modification of ComX (putative). Is required for ComX production.
comP	Membrane-bound receptor for ComX. Upon activation, this histidine kinase autophosphorylates and donates phosphate to ComA.
comA	Upon activation by ComP, this transcriptional activator regulates the expression of select genes.

Quorum sensing control *of Pseudomonas aeruginosa*





N-acyl homoserine lactones

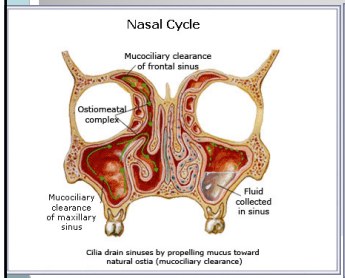
LasI/LasR
RhlI/RhlR

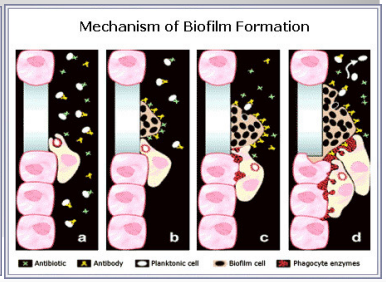
Erwinia carotovora, *Serratia* species

Why is Chronic Sinusitis so Hard to Treat?

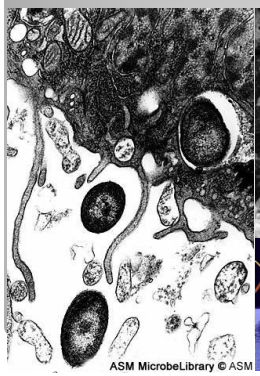
- Chemical signaling induces biofilm that effects
  - Low metabolism of bacteria, protect them from immune and antibiotic.

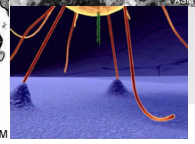
xylitol





Pseudomonas aeruginosa Displays Multiple Phenotypes during Development as a Biofilm





Do All strains have the gene transcription depend on cell density-dependent QS ?

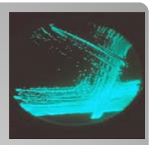
P. aeruginosa from canine otitis externa

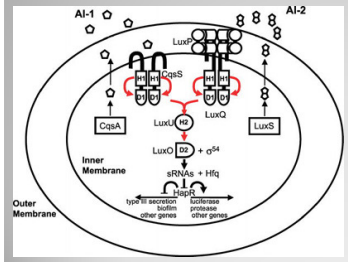
Non-mucoid canine strains exhibites elastase deficiency phenotype

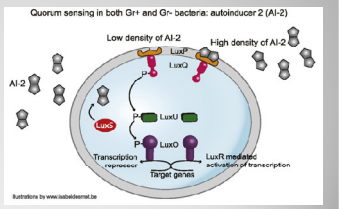
This phenotype is strain-specific.

It is quite stable upon passage.

QUORUM SENSING IN *Vibrio cholera*: A HYBRID HOMOSERINE LACTONE/TWO-COMPONENT SIGNALING CIRCUIT





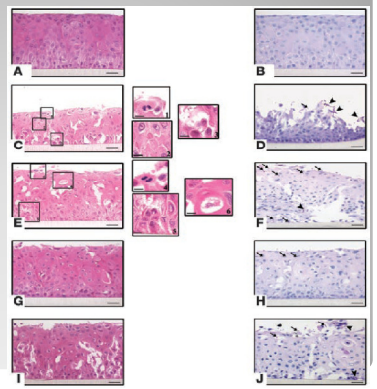


Fungi: *Candida* and *Saccharomyces* talks

- Media:
  - Tyrosol → Biofilm, Germ tube
  - Farnesol → Yeast form, True hyphae

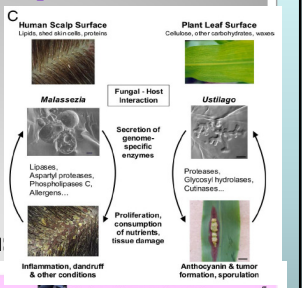
Targeted gene deletion in *Candida parapsilosis* demonstrates the role of secreted lipase in virulence

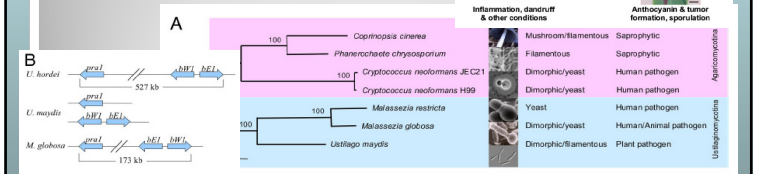
- (**A** and **B**) Uninfected control tissues, (**C** and **D**) WT, (**E** and **F**) heterozygous mutant *CpLIP1-2/Δcplip1-2*, (**G** and **H**) homozygous mutant *Δcplip1-2/Δcplip1-2*, and (**I** and **J**) reconstituted *Δcplip1-2/CpLIP2*.
- **C** and **E** show apoptotic cells; middle insets in **C** and **E** show intercellular edema; bottom inset in **C** shows cleft formation and tissue separation; and bottom inset in **E** shows vacuolization.

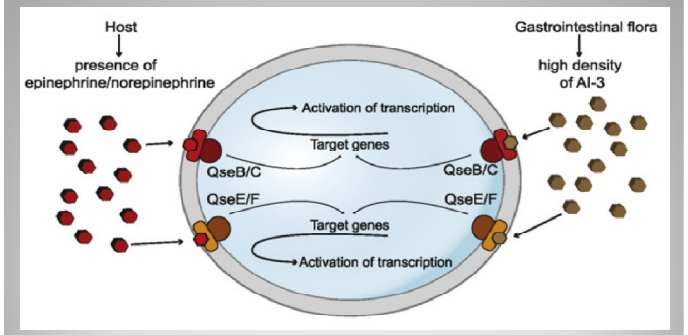


Dandruff-associated Malassezia genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. PNAS USA November 13, 2007

- Genome
- secretory proteome
- expression of selected genes
- *Malassezia globosa* - *Ustilago maydis*
- *Candida albicans* - extracellular hydrola







Quorum sensing beyond bacterial borders: autoinducer 3 (AI3)

Food poisoning caused by Salmonellosis and EHEC

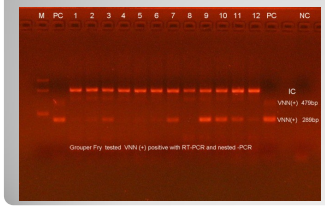
- Epinephrine/norepinephrine analog characterized auto inducer 3 (AI-3) : misuse signal
  - Supplier iron
  - QseC AI-3 sensor
  - Play a role in interkingdom signalling
- eg. Sudden burst of *Salmonella* excretion by stressed pigs.

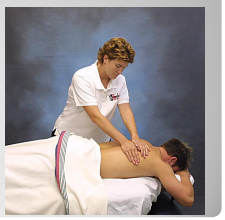
Clinical significance of QS in Veterinary pathogens

- *S. aureus*
- *S. pseudintermedius*
- *Pseudomonas aeruginosa*
- *Salmonella*
- Other Vet Pathogens

Future perspectives of QS

- QS signal molecule detection as a diagnostic tool?
- QS inhibition as therapeutic tool?

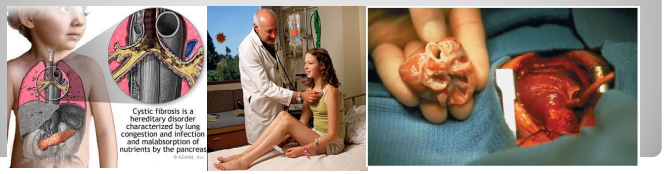




QS signal molecule detection as a diagnostic tool?

- Biomaker for
  - Onset of pathogenecity (Wu *et al.*, 2007)
  - Follow-up of infection (Kumari *et al.*, 2008)

Pseudomonas aeruginosa and *Burkholderia cepacia* of cystic fibrosis



QS inhibition as therapeutic tool?

- *Bacillius* sp. harbours AHLase. (Dong et al., 2001)
- Human cell line (Chun et al., 2004)
- Macrolide attenuates bacterial pathogenecity.
- LED209 inhibits the QseC (mediated virulence gene activiation) (Rasko et al., 2008)
- QS inhibitor for biofilm formation in *P.aeruginosa, S. aureus* and *Salmonella* Typhimurium (Janssens et al., 2007)
- AHL analogues reduces the virulence gene of *A salmonicida* and *V anguillarum* (Rasch et al., 2004, 2007)

Biofilm disrupted by Sugar Ester

- Lauroyl glucose coated on polystyrene and glass surfaces :
  - Against *C. albicans, C. lypolytica, P. aeruginosa* and *P. aureofaciens*.

Preventing of Biofilm

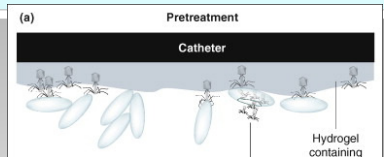
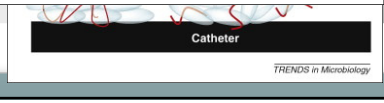


Table 1. Examples of the effect of phage on biofilms

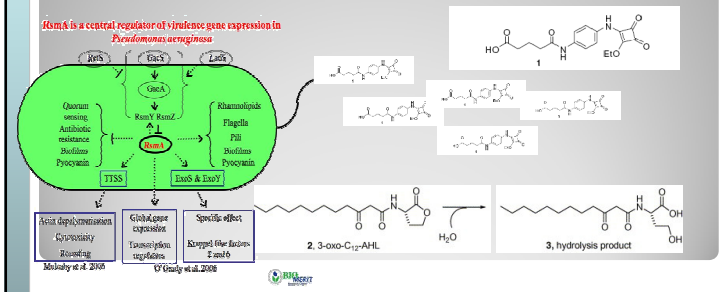
Biofilm bacterium	Phage	Experimental approach[a]	Results	Refs
E. agglomerans 53b	SF153b	24 h biofilms (~10^8 CFU per cm^2) treated with 10^{10} PFU per ml of phage for 15 min	3 $\log_{10}$ reduction in biofilm viable count	[19]
E. coli 3000XIII	T4	28 h biofilms treated with 10^8 or 10^{10} PFU per ml phage for 30 min to 8 h	6 $\log_{10}$ reduction in biofilm viable count within 5 h	[20]
E. coli K12	T4	72 h biofilms treated with a phage:cell ratio of 10:1 or 100:1 for 30 min	3 $\log_{10}$ reduction in biofilm viable count. Regrowth after 3 h. Phage:cell ratio of 100:1 resulted in greater change in the biofilm structure.	[24]
E. coli O157:H7	KH1	72 h biofilms treated with 10^7 PFU per ml of phage for 1–4 days	1.2 $\log_{10}$ reduction in biofilm viable count	[75]
E. coli TG1	T7 and $T7_{DspB}$	24 h biofilms treated with 10^1-10^5 PFU per ml of phage for 0–50 h	Greater than 3 $\log_{10}$ reduction in biofilm viable count over 48 h. T7 phage engineered with dispersin B gene was most effective	[76]
P. aeruginosa NCIMB10548	F116	Biofilms of different ages treated with phage:cell ratio of 100:1 or 1000:1 for 24 h	100:1 phage:cell caused 1 $\log_{10}$ reduction in biofilm viable count; 1000:1 phage:cell caused 2 $\log_{10}$ reduction	[28]
Pseudomonas fluorescens ATCC 27663	ΦS1	5 day biofilms treated with 10^9 PFU per ml phage for 0–200 min at different temperatures	Up to 84% reduction in biofilm biomass after 200 min, with greatest reduction at 26 °C	[77]
Staphylococcus epidermidis (multiple strains)	Staphylococcus phage K	24 h biofilms treated with 10^8 PFU per ml phage for 24 h	Significant reduction in biofilm optical density for several strains	[42]



Donlan RM., 2009 **Trends in Microbiology**

Immunotherapy using antibody against AHL quorum sensing

- *AI of Pseudomonas aeruginosa is blocked with squaric monoester monoamide hapten*



คนไทยลองตั้งใจทำอะไร ไม่แพ้ชาติใดในโลก

Thank you for your attention