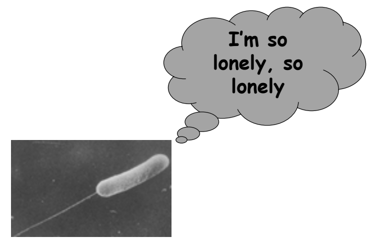
Bacterial Cell-Cell Communication,

or

Quorum Sensing

Previous paradigm:

Bacteria are asocial creatures, living a unicellular existence, responding only to chemical and physical signals from the environment.

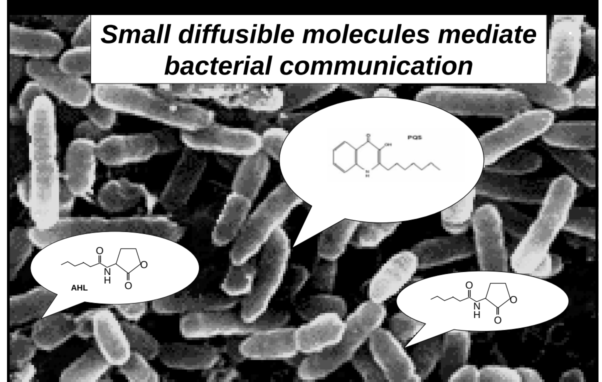


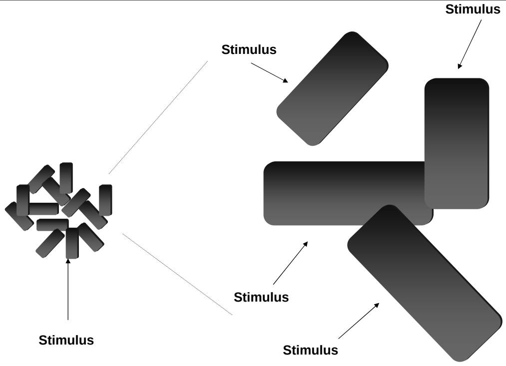
"It is perfectly possible to imagine a rather boring universe without sex, without hormones, and without nervous systems; a universe peopled only by individual cells reproducing *ad infinitum*. This universe, in fact, exists. It is the one formed by a culture of bacteria."

--Dr. Fancois Jacob, 1973

You underestimate me.

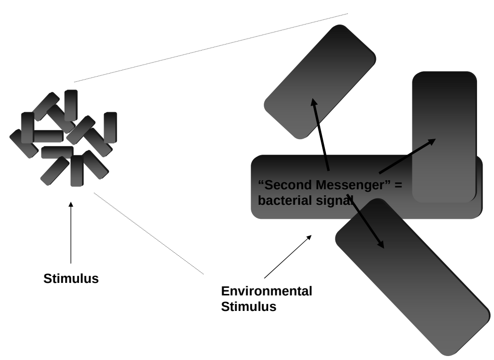
Small diffusible molecules mediate bacterial communication



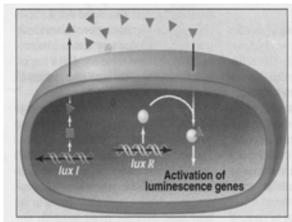


In the absence of communication, each cell has to <u>individually</u> sense and respond to environmental cues

---



Bacterial signaling and quorum sensing allows bacteria to act similarly to multicellular organisms

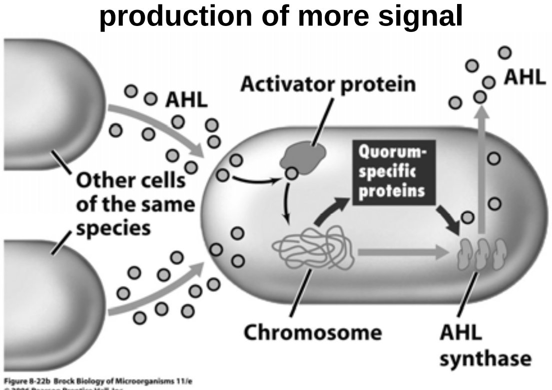


What does bacterial cell-cell signaling accomplish for the population?

-Coordination of behavior

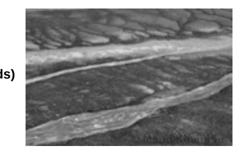
-Quick response to environmental stimuli

Autoinduction: signal stimulates production of more signal

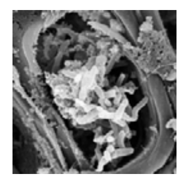


What behaviors are coordinated?

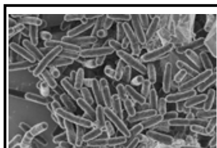
1. Adaptation to environmental conditions
   a. Availability of nutrients
   b. Stress (harsh conditions, toxic compounds)
   c. Proximity of host
2. Defense against other microorganisms
3. Expression of virulence genes during host infection
   a. First, escape host immune response. Avoid wasting expensive resources on virulence traits, unless they will result in successful infection
   b. Next, overwhelm host all at once by coordinated expression of virulence genes
4. Coordination of symbiosis: ensure benefit to kin, not cheaters



Bacterial mat in Yellowstone



Xylella fastidiosa in xylem



Pseudomonas aeruginosa in lungs
~ 5% of genome responds to homoserine lactone signal (350 genes)

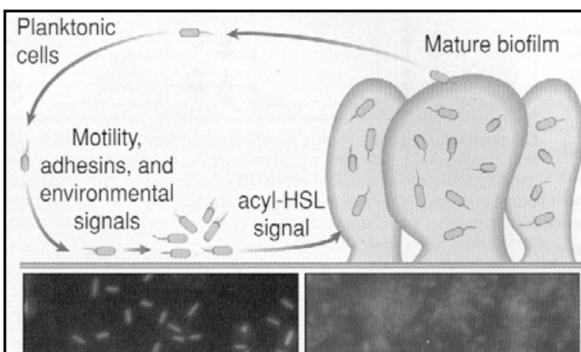
"If the bacteria had acted as independent assasins rather than as an army the immune system would have wiped them out" …Dr. Bonnie Bassler

"A multitude of bacteria are stronger than a few, thus by union are able overcome obstacles too great for few."

... Dr. Erwin F. Smith, 1905 (Father of Plant Bacteriology)

This doesn't sound like an antisocial organism, does it? In fact, it sounds almost… multicellular!

(r = 1 in multicellular and clonal populations; does Hamilton's rule apply?)



Construction of a biofilm. Free (planktonic) bacteria assemble on a surface (**left**). Cell-to-cell communication then induces the formation of multicellular pillars and columns (**right**).

How do HSLs affect bacterial biofilms?

-help ammonia oxidizing bacteria recover more quickly from ammonia starvation

-allow thicker, denser biofilms of *Pseudomonas aeruginosa* (pathogen infecting lungs of cystic fibrosis patients)

-make bacterial populations more resistant to bacteriostatic (detergents) and bactericidal (antibiotics) substances – physical barrier

-trigger antibiotic production by plant pathogenic bacteria to exclude competitor species as biofilm community degrades host tissues and free up nutrients

Are there more functions? Remember: emergent properties…

We know that HSL signaling is important in mediating some host-bacterial interactions… but what other functions might HSLs have?

One cannot always predict the properties (utility) of a biological system just by describing it *in vitro*.

Properties emerge in context of environment, population, etc.: "emergent properties"

Cannot always be predicted through logical reasoning because we don't fully understand the ecology

For example, we now know that there is *cross-species and cross-genus* signaling by HSLs… what importance might this have ecologically?

A well-known example of an emergent property:

Hemoglobin mutation = morphologically deformed blood cells (sickle cell anemia)
Hemoglobin mutation = protection against malaria

How important is quorum sensing to bacteria?

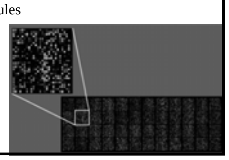
Pseudomonas aeruginosa:
(*luxI*, *rhlI* mutants examined)

6% of ~6,000 genes are induced or repressed by the quorum sensing signals in this species.

Escherichia coli
(*luxS* mutant examined)

5.6% of the genome (242 of 4,290 genes) is induced or repressed significantly by the *luxS* product AI-2

Functions of genes involved:
cell division
DNA processing
morphological changes
metabolism of small molecules
onset of stationary phase
signal transduction genes



What phenotypes are regulated by quorum sensing via homoserine lactone signals?

Bioluminescence (*Vibrio fischeri, V. harveyi*)

Antibiotic biosynthesis (*Erwinia. caratovora* subs. *carotovora, Streptomyces griseus, Pseudomonas fluorescens, P. aureofaciens*)

Pathogenicity (*E. carotovora, Pantoea stewartii, Pseudomonas aeruginosa, Ralstonia solanacearum, Xanthomonas campestris*)

Plasmid conjugal transfer (*Agrobacterium tumefaciens*)

Competence (*Bacillus subtilis*)

Biofilm formation (*P. aeruginosa*)

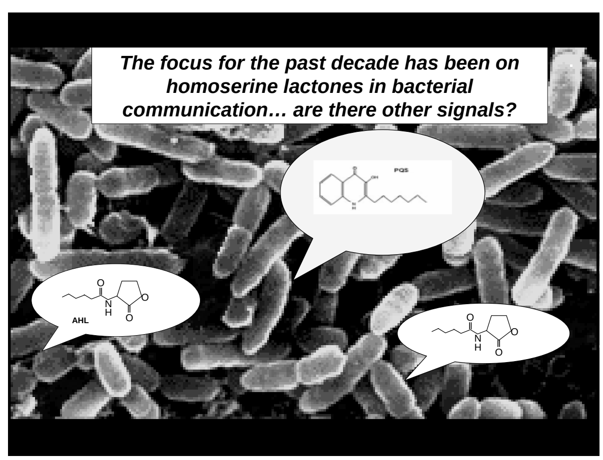
Symbiosis (*Rhizobium etli, R. leguminosarum bv. viciae*)

Very important in plant and human disease, integral in many eukaryotic-host associations.

Phenotypes regulated by homoserine lactone signals often have to do with interations OUTSIDE the bacterial cell

Bioluminescence	(***Vibrio fischeri***, *V. harveyi*)
Antibiotic biosynthesis	(***Erwinia. caratovora* subs. *carotovora***, *Streptomyces griseus, Pseudomonas fluorescens, P. aureofaciens*)
Pathogenicity	(***E. carotovora, Pantoea stewartii, Pseudomonas aeruginosa, Ralstonia solanacearum, Xanthomonas campestris***)
Plasmid conjugal transfer	(***Agrobacterium tumefaciens***)
Competence	(*Bacillus subtilis*)
Biofilm formation	(***P. aeruginosa***)
Symbiosis	(***Rhizobium etli, R. leguminosarum bv. viciae***)

Very important in plant and human disease, integral in many eukaryotic-host associations: bolded species rely on HSL-mediated phenotypes in interactions with eukaryotic host.



Not all bacterial cell-cell signals are homoserine lactones!

Quorum sensing signal diversity:

- **homoserine lactones: usually >1 per species (e.g. *V. fischeri, Pseudomonas aeruginosa*)** — Generated by LuxI homologs
- **peptide pheromones (e.g. Gram+ bacteria such as *Bacillus subtilis*)**
- **polyketides (e.g. *Pseudomonas fluorescens*)**
- **quinolones (e.g. *Pseudomonas aeruginosa*)**
- **furanones (e.g. *V. harveyi*)**
- **mixture of amino acids (*Myxococcus xanthus*)**
- **hydroxypalmitic acid methyl ester (*Ralstonia solanacearum*)**
- **subinhibitory concentrations of antibiotics!!**

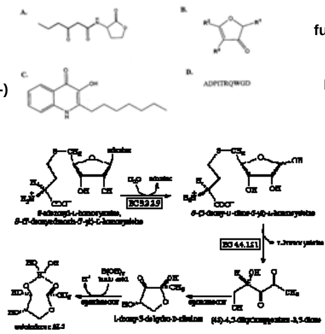
(peptide pheromones through subinhibitory concentrations of antibiotics: Other synthetic pathways)

Signal diversity

HSL (G-) — **furanone**

Quinolone (G-) — **Peptide (G+)**

AI-2 (Gram- and Gram+; bacterial Esperanto



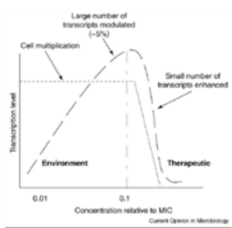


Table 1

Phenotypic effects of SI antibiotics.

Antibiotic and action	Class	Concentration	Responsive genes	Method and Ref
B. subtilis				
Protein synthesis — chloramphenicol		0.4 × MIC	+ Transport/binding + Ribosomal proteins − Metabolism of AA[a]s + Metabolism of carbohydrates	Microarray [13]
Gentamicin	Amino-glycoside	0.4 × MIC	− Transport/binding	Microarray [13]
Erythromycin	Macrolide	0.5 × MIC	+/− Transport/binding − Metabolism of carbohydrates +/− Ribosomal proteins − Metabolism of AAs − Purine and pyrimidine biosynthesis	Microarray [13]
Cell wall biosynthesis inhibitor — bacitracin		0.2 × MIC	+ *sigB* (general stress) and *sigM* (ECF) regulon	Microarray [20]
Streptococcus pneumoniae				
Protein synthesis inhibitor — puromycin	–	Sub lethal	+ Heat shock − AA biosynthesis, AAR[b]s + Ribosomal proteins (weakly) + Purine nucleotide biosynthesis	Microarray [24]
Protein synthesis inhibitor — tetracycline	Tetracycline	Sub lethal	− AA biosynthesis, AARs + Ribosomal proteins (weakly) + Purine nucleotide biosynthesis	Microarray [24]
Protein synthesis inhibitor — chloramphenicol		Sub lethal	− AA biosynthesis, AARs + Ribosomal proteins (weakly) + Purine nucleotide biosynthesis	Microarray [24]
Protein synthesis inhibitor — erythromycin	Macrolide	Sub lethal	− AA biosynthesis, AARs + Ribosomal proteins (weakly) + Purine nucleotide biosynthesis	Microarray [24]

Davies et al., 2006. Curr. Opin. Microbiol. 9: 445

Table 1

Phenotypic effects of SI antibiotics.

Antibiotic and action	Class	Concentration	Responsive genes	Method and Ref
E. coli				
Bac7(1-35)	Antimicrobial peptide	0.25 × MIC	− Maltose, ribose transport system + Osmotic stress (uptake of osmoprotectant glycine, betaine and proline) + hns + Prophage genes	Macroarray [17]
Protein synthesis inhibitor — 4-azaleucine		Sub lethal	+ Heat shock + AA biosynthesis + Ribosomal proteins	Microarray [23]
Protein synthesis inhibitor — mupirocin		Sub lethal	+ AA biosynthesis	Microarray [23]
Protein synthesis inhibitor — kasugamycin		Sub lethal	+ Ribosomal proteins + Transporters − Carbon metabolism − rpoS regulon	Microarray [23]
Protein synthesis inhibitor — puromycin		Sub lethal	+ ribosomal proteins − rpoS regulon − Carbon metabolism − Iron metabolism	Microarray [23]
***E. coli* O157**				
Gyrase inhibitor — norfloxacin	Fluoroquinolone	0.8 × MIC	+ Prophage genes − LEE genes − Membrane protein genes − Protein biosynthesis genes − AA biosynthesis genes	Microarray [14]

Table 1 (Continued')

Antibiotic and action	Class	Concentration	Responsive genes	Method and Ref
S. typhimurium				
Cell membrane biosynthesis inhibitor — polymyxin	Cationic antimicrobial peptide	Sub lethal, 30 min treatment	+ rpoS regulon + phoP regulon + Exopolysaccharide biosynthesis genes + AMP resistance genes − Invasion genes − Flagellar genes	2DGE and microarray [21]
RNA polymerase inhibitor — rifampicin	Rifamycin	0.4 × MIC	+ Virulence genes involved in intracellular survival − Flagellar genes and virulence genes involved in invasion	Lux-reporter library [22], Yim et al., unpublished

[a] AA, amino acid.
[b] AAR, aminoacyl-tRNA synthetase.

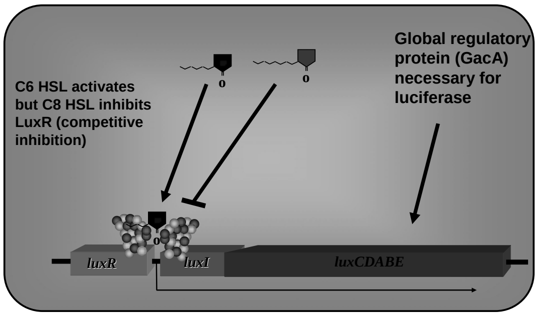
Table 2

Functional groups of genes affected by SI antibiotics

Antibiotic	Organism	Effect	References
Tetracyclines	*Bacteroides sp*	Enhanced gene transfer (conjugation of antibiotic resistance genes)	[57]
	S. epidermidis	Stimulation of bacterial adhesion	[58]
	Streptococcus sp	Changes in exoprotein secretion	[59]
β-lactams	*Staphylococcus sp*	Decreased biofilm formation	[60]
Cerulenin	*S. aureus*	Inhibition of protein secretion	[61]
Aminoglycoside	*P. aeruginosa*	Increased biofilm formation	[62]
	S. pneumoniae	Increased mutation frequency	[63]
Fluoroquinolones	*E. coli*	Reduced hemolytic activity	[64]
		Induction of colicin synthesis	[65]
	S. aureus	Increased adhesion	[66]
	S. pneumoniae	Increased mutation frequency	[63]
	Mycobacterium fortuitum	Increased mutation frequency	[67]
Macrolides	*Mycobacterium avium*	Decreased biofilm formation	[68]
	P. aeruginosa	Inhibition of quorum sensing (virulence suppression)	[69]
Lincosamides	*Bacillus fragilis*	Altered cell morphology and increased DNA fragmentation	[70]
	S. aureus	Changes in exoprotein expression	[59]
Oxazolidinone	*S. aureus*	Decreased secretion of virulence factors	[71]
Mupirocin	*P. aeruginosa*	Reduced biofilm formation	[72]
		Reduced flagellin expression	[73]
Rifampicin	*E. coli*	Reduced toxin secretion	[74]

Davies et al., 2006. Curr. Opin. Microbiol. 9: 445

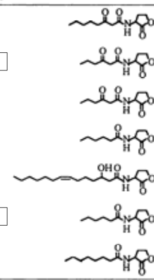
HSLs can act as opposing signals



Many Gram negative bacteria produce *N*-acyl-homoserine lactone signals

By 1991 more quorum sensing systems were discovered

Table 1 Examples of plant-associated bacteria known to utilize AHL signals and name*



Bacterium	LuxI/LuxR homolog	AHL sidechain and name*		AHL-regulated properties
A. tumefaciens		*N*-3-oxooctanoyl	(OOHL)	Ti plasmid conjugal transfer
E. caratovora		*N*-3-oxohexanoyl	(OHHL)	Extracellular virulence factors, carbapenem
P. stewartii		*N*-3-oxohexanoyl	(OHHL)	Extracellular polysaccharide capsule
P. aureofaciens		*N*-hexanoyl	(HHL)	Phenazines
R. leguminosarum		*N*-3-hydroxy-7-*cis*-tetradecanoyl	(HTDHL)	Rhizome interactions, root nodulation
R. solanacearum		*N*-hexanoyl	(HHL)	*aidA*?
		N-octanoyl	(OHL)	

Strains, genes and gene products are described in the text. *Name of the moiety attached to the homoserine lactone ring. Common abbreviations are given in parentheses.

Signals allow for crosstalk

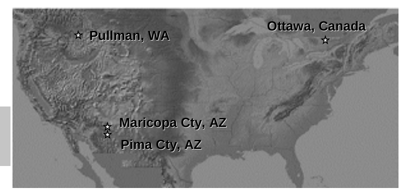
1. **Cross-talk or cross-activation by HSLs of other strains**

50% of bacteria isolated from wheat roots produced HSLs
Range: 106 isolates crossing 7 genera
8% could activate phenazine operon of *P. aureofaciens*

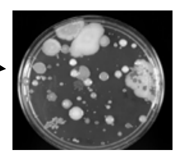
2. **The *luxS* gene product, AI-2 is a generally recognized signal**

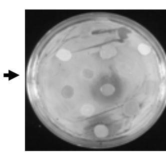
At least 12 genera of Gram-negative bacteria produce AI-2
Unlike HSLs, not species-specific: "bacterial esperanto"
HSL allows bacteria to sense population density; AI-2 allows them to sense community density

Previously: 1) AHL's required for *phz* expression
2) isogenic AHL donor could complement a *phzI* mutant



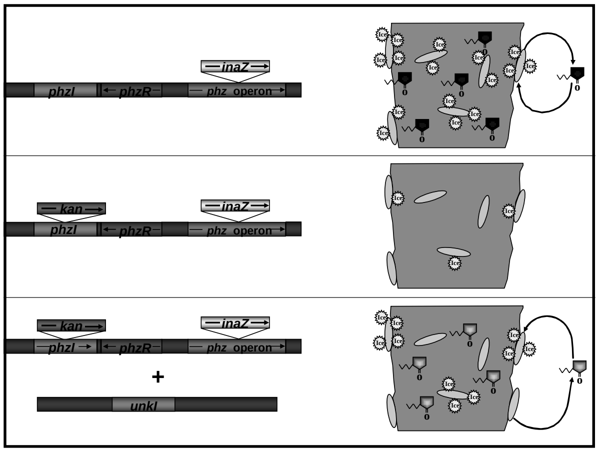
What about the rhizosphere community?



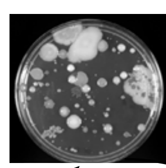


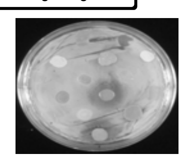
~ 8% activated phenazine gene expression

Lawn of 30-84I

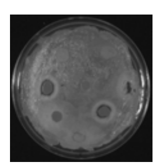


Cross- communication using Negative signaling





Lawn of 30-84I
(PhzI-)
'Good Neighbors'
Positive communication



'Bad Neighbors'
Negative Communication
Lawn of 30-84 (WT)

Cross-Communication

- The demonstration that signal produced by one strain of bacteria could alter the expression of QS regulated genes in another strain of bacteria altered the model of QS regulation.
- It is the quorum of bacterial *signals* (does not have to be of isogenic origin) that the bacteria senses—not the number of isogenic bacterial cells.
- Need to consider all the signal producing members of the community when thinking about the regulation of of QS mediated traits and ultimately the ecology of interactions in mixed communities.

Signal degradation

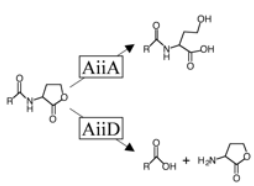
HSL signal must be turned over to dampen response.

abiotic: diffusion
abiotic: alkali
self-degradation: AI-2 (product of LuxS)
non-self: AiiA, AiiD: metallohydrolase enzymes of *Bacillus spp.*

Bacillus is Gram+; HSLs are only found in Gram- bacteria. Why might *Bacillus* produce HSL-degrading enzymes?

Other bacterially produced enzymes that degrade HSLs are likely.

Signal degradation



Ring opening by cleavage of ester bond

Hydrolysis of amide link between HSL and acyl side chain

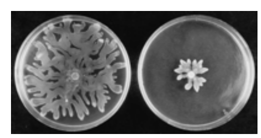
Expression of a *Bacillus aiiA* gene in *P. aeruginosa* PAO1: Potential for antibiotic?

-reduced the amount of quorum sensing signal
-reduced swarming
-decreased production of several virulence factors and cytotoxic compounds
elastase
rhamnolipids
hydrogen cyanide

Important in setting up lung infections

However, no effect was observed on:
flagellar swimming
bacterial adhesion to surfaces

Reimmann et al. 2002. Microbiology 148:923-32



Heterologous expression of *Bacillus* spp. AiiA lactonases in *Burkholderia thailandensis*

-reduced AHL accumulation
-affected motility
-slowed growth
-prevented the beta-hemolysis of sheep erythrocytes

--Ulrich, RL. 2004. Appl. Environ. Microbiol. 70: 6173-6180

Signal mimicry

Certain molecules inhibit (antagonize) the perception of HSL by competing for LuxR

-Other HSLs from same or other organisms

-furanones secreted by macroalga *Delisea pulchra* probably evolved to disrupt colonization of seaweed

-diketopiperazines (cyclic dipeptides) produced by bacteria, fungi

-unidentified compounds from pea and other higher plants

Couldn't use pharmaceutically because some HSLs affect immune and cardiovascular systems

Pharmaceutical value

Signal mimicry

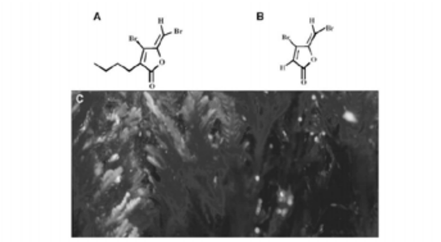
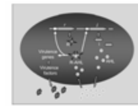


Fig. 1. From algal metabolite to *Pseudomonas* drug. (A) Compound 2, a natural furanone compound isolated from (C) *D.pulchra*. (B) compound C-30, a synthetic furanone with enhanced QSI activity.

The EMBO Journal Vol.22 No.15 pp.3803-3815, 2003

Exploiting quorum sensing for antimicrobial therapy

- **We know QS regulates the virulence in many pathogenic bacteria.**
- **Can we exploit this fact and look for quorum sensing inhibitors for antimicrobial therapy??**



- **Strategy I : Quorum quenchers inactivating AHL signals already identified:**
  - **AHL lactonases (*aiiA from Bacillus* sp)**
  - **AHL acylases (*aii*D from *Ralstonia* sp)**

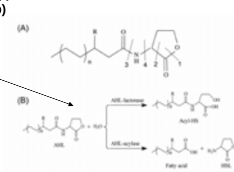


Fig. 2. The general structure of AHL signals and enzymatic degradation products. (A) The AHL structure and its possible enzyme cleavage sites (see text for discussion of potential enzymes); (B) the corresponding degradation mechanisms of AHL-lactonase and AHL-acylase.

- **Strategy II : Blocking the AHL receptor protein by using halogenated furanone compounds from marine algae (*Delisea pulchra*)**
- **Mechanism??**