MarBEF course on chemical ecology in Tjärnö / Sweden

day one

microbiology

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Bremen

Germany

www.mpi-bremen.de

introducing myself

Jens Harder

diploma in chemistry

PhD in protein biochemistry

postdoc in enzymology and molecular biology

(2 wonderful years in Stockholm
but I did not learn Swedish)

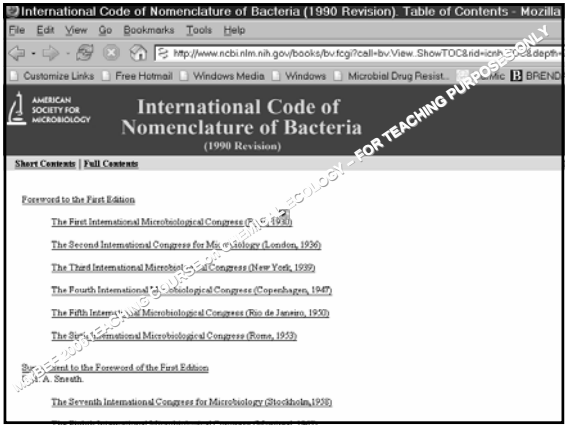
habilitation in microbiology

head of DIVERSITY group

(enzymology of monoterpenes

diversity of cultivable microorganisms

molecular ecology of species diversity)



Microbiology works with strains
- a strain consists of the descendants of a single cell

A species consists of a collection of strains with a DNA-DNA relatedness of over 70 % (hybridization experiment)

A DNA-DNA relatedness of 70 % corresponds to an average nucleotide identity of 94% in conserved genes of the genome (60-90 % of the genome)

Konstantinidis and Tiedje, PNAS 2005

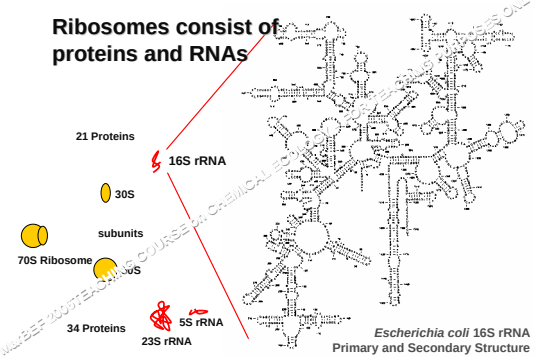
Human and chimpanzee have an ANI of 98.5 %

Nature 437, 69-87 (1 September 2005) doi:10.1038/nature04072

DNA-DNA relatedness studies are demanding, first identification of a new isolate with 16S rRNA gene sequencing

16S rDNA gene has around 1560 bp, conserved and variable regions

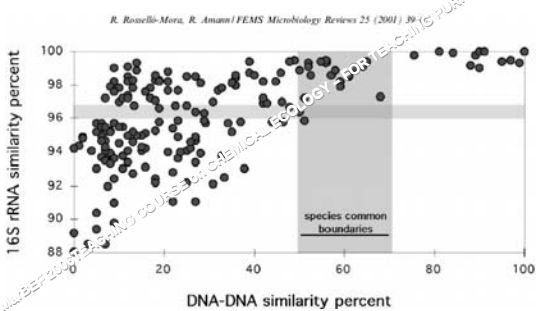
Ribosomes consist of proteins and RNAs



Escherichia coli 16S rRNA
Primary and Secondary Structure

Correlation between 16S rRNA similarity and DNA-DNA-hybridization:

<97% rRNA similarity strongly suggests two species, > 97% ????



Safe criterion: less than 97 % identity over full 16S rRNA gene sequence is considered as separate species (underestimation of true diversity)

How many microbes and microbial species are in nature?

Microbes are the unseen majority!

Marine Water	10^8-10^9/l	ca. 10^{29}
Marine Sediment	10^9/g	ca. 3x 10^{29}
Intestinal Tracts	10^{11}/g	ca. 10^{25}
Deep Subsurface	10^2-10^8/g	ca. 10^{30}

Prokaryotes" account for 25-50% of the global biomass!

W.B. Whitmann et al. PNAS 1998

MA-BEF 2008 TEACHING COURSE ON CHEMICAL ECOLOGY – FOR TEACHING PURPOSES ONLY

Biologists have described more than 1.5 Mio Species...

< ...but there are today only about 6000 validly described prokaryotes

So many prokaryotes, and only 6000 species?

Description of a new species according to the

International Code of Nomenclature of Bacteria

is a major task (1 species / man-year)

(1000 16S rRNA genes / man-year)

Not everything is easily culturable

Many environmental bacteria may be difficult to isolate....

Habitat	Cultivation efficiency [%]	Reference
Marine water	0.001 to 0.1	Ferguson *et al.* 1984
Lake water	0.1 to 1	Staley & Konopka 1985
Estuary	0.1 to 3	Ferguson *et al.* 1984
Activated sludge	1 to 15	Wagner *et al.* 1993
Sediment	0.25	Jones 1977
Soil	0.3	Torsvik *et al.* 1990

.... <1000 species of marine bacteria have been validly described!

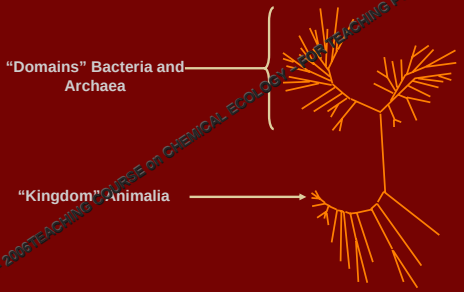
So many prokaryotes, and only 6000 species?

Description of a new species according to the International Code of Nomenclature of Bacteria is a major task (1 species / man-year)
(>1000 16S rRNA genes / man-year)

Not everything is easily culturable

So what do we learn from molecular ecological studies mainly based on 16S rRNA gene sequences?

Tree of Life



Prokaryotes

1994 - 13 divisions
(all cultured)

1997 - 36 divisions
24/12

2003 - 53 divisions
26/27

2004 - 80 divisions
26/54

Status of the Microbial Census

September 2003

78166 partial 16S rRNA gene sequences

(June 30, 2006: 243909 16S rRNA gene sequences

current rate 100 000 new sequences/year)

alignment yielded 56215 sequences

analysis for < 97 % identity yielded

35280 operational taxonomic units (OTUs)

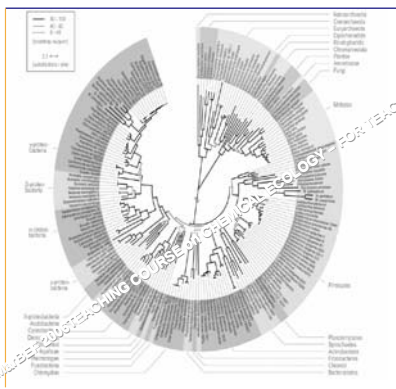
Chao1 richness estimates 325040 OTUs

Schloss and Handelsman, MMBR 68, 686 (2004)

Other diversity estimates

DNA-DNA hybridization studies of environmental DNA

estimates 10 000 000 till 1 000 000 000 species



This is now confirmed by genome-based tree reconstructions

Bacteria

Archaea

Eucarya

Ciccarelli ...& Bork. 2006. Towards automatic reconstruction of a highly resolved tree of life. Science 311:1284-87.

High density and high diversity of prokaryotes

Challenge and chance for chemical ecology

Focus on functional aspects

1. principles of cultivation and isolation
2. chemical communication between bacteria
3. considerations on a bioluminescent bioassay
4. cultivation-independent molecular studies
5. porifera-microbe interactions

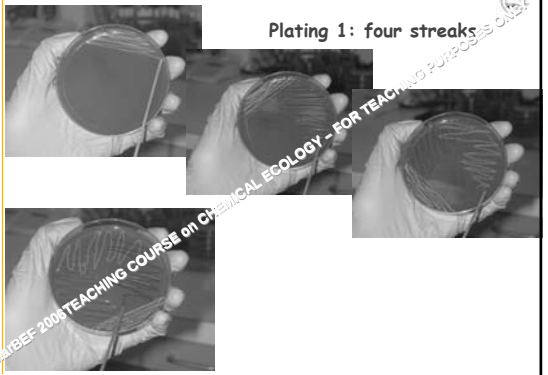
Principles of cultivation and isolation: variables of a game

Physical separation
- Separation by ultrasound, ...
- Dilution in liquid series or on plates

One microbe per culture tube or per cm² plate

Physiological separation (Selection for niches)
- energy source
- electron donor
- carbon source
- supply of elements C, N, S, P, K, Mg, Ca, Na, Cl, Fe
- supply of trace elements Mn, Co, Cu, Mo, Zn, Ni, V, Se, W, B
- growth factors (vitamins, nucleotides, ...)
- pH, osmolarity and ionic composition
- temperature, hydrostatic pressure
- oxygen pressure, redox-potential
- toxic compounds (antibiotics, trace metals, hydrocarbons, ...)
- growth rate and substrate turnover rates

Plating 1: four streaks



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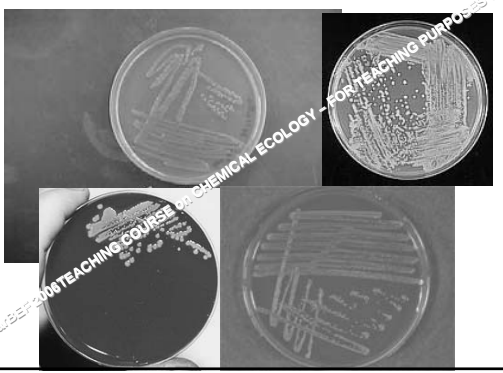
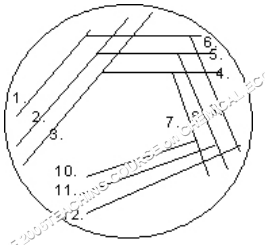
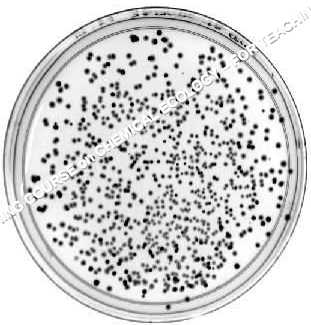


Plating 2: twelve streaks => dilution gradient



Plating 3: liquid dilution series and spread plating



High density and high diversity of prokaryotes

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Quorum sensing

Being part of a community

More information e. g. at http://www.nottingham.ac.uk/quorum/index.htm

Bioluminescence

Bioluminescent bacteria can be cultured on both liquid and solid medium. When they have reached a high cell density their light emission can be visualised by the naked eye.

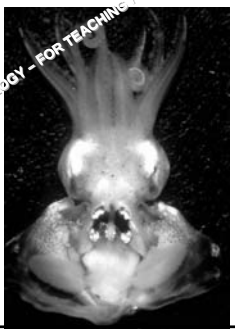
Vibrio fischeri

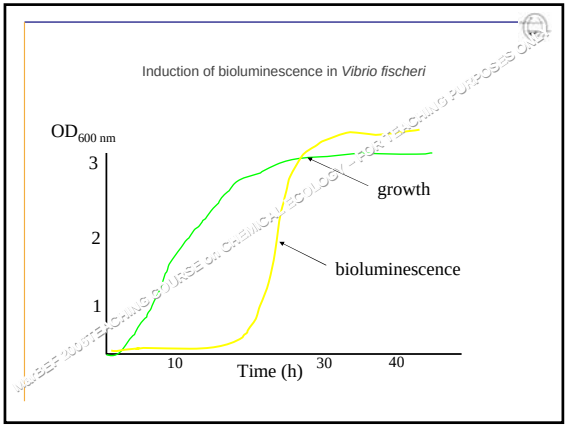
***Photobacterium* spp.**

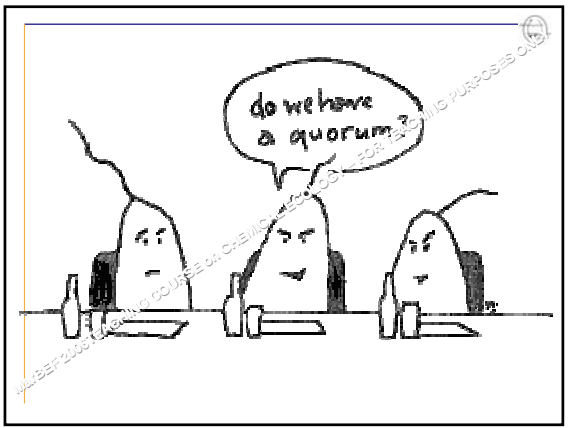
Vibrio bioluminescence

***Vibrio fischeri* inhabits the open sea (in low densities), as well as the light organs of squid (in high densities)**

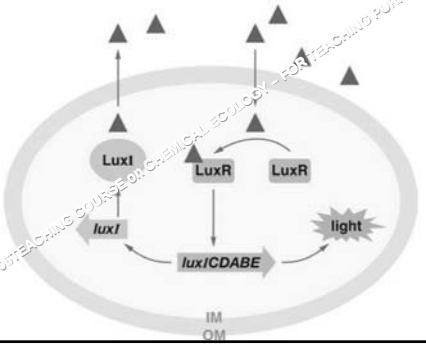
luminescence occurs only at high bacteria cell concentrations (above the quorum)




Induction of bioluminescence in *Vibrio fischeri*
$OD_{600\ nm}$
3
2
1
growth
bioluminescence
10
30
40
Time (h)

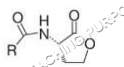

do we have a quorum?

Quorum Sensing Circuit



Acyl-homoserine lactones (AHL)

Core Molecule



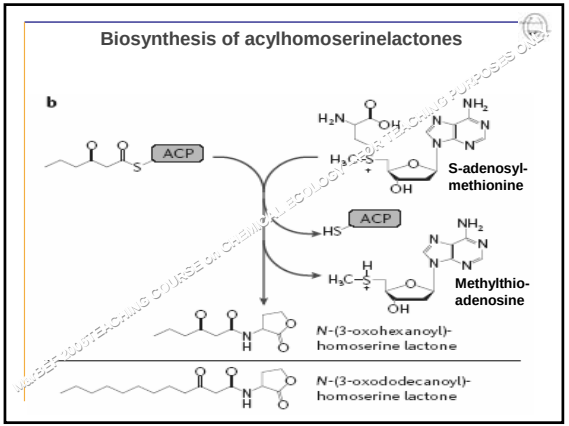
R groups:

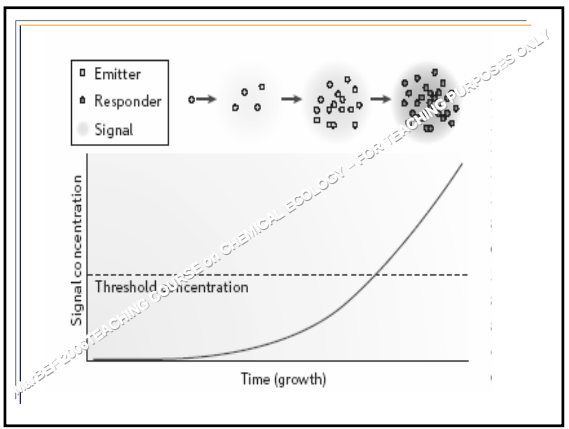
LuxI *(V. fischeri)*

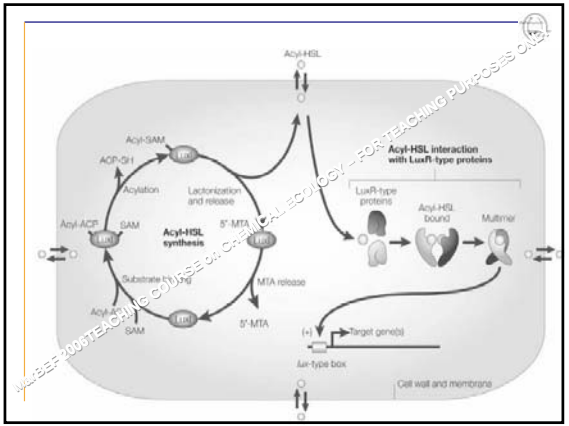
LuxM *(V. harveyi)*

RhlI *(P. aeruginosa)*

LasI *(P. aeruginosa)*


Biosynthesis of acylhomoserinelactones
b
ACP
S-adenosyl-
methionine
ACP
Methylthio-
adenosine
N-(3-oxohexanoyl)-
homoserine lactone
N-(3-oxododecanoyl)-
homoserine lactone

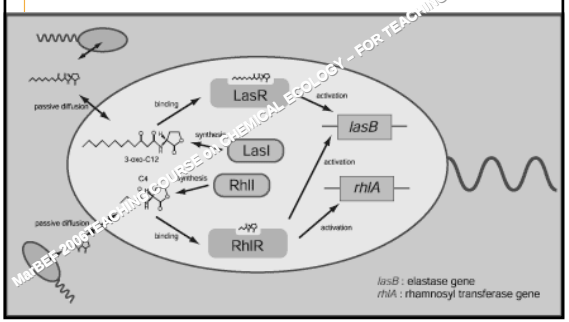

Emitter
Responder
Signal
Signal concentration
Threshold concentration
Time (growth)



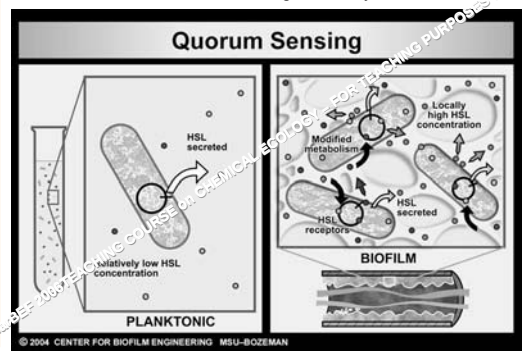
Two quorum sensing systems in *Pseudomonas aeruginosa*

hierarchical structure: *las* controls positively

(at least partially) the *rhl* system



Biofilms are sites of high cell density

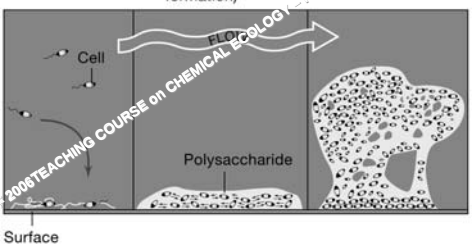


Biofilms

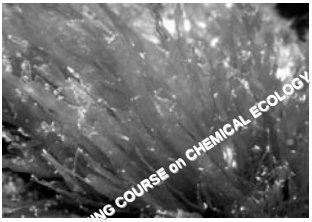
Attachment (adhesion of a few cells to a suitable solid surface)

Colonization (intercellular communication, growth and polysaccharide formation)

Development (more growth and polysaccharide)



Ulva intestinalis zoospores uses the quorum sensing signal to identify settling sites



Algal sensing as indication of hard bottom substrata for anchoring

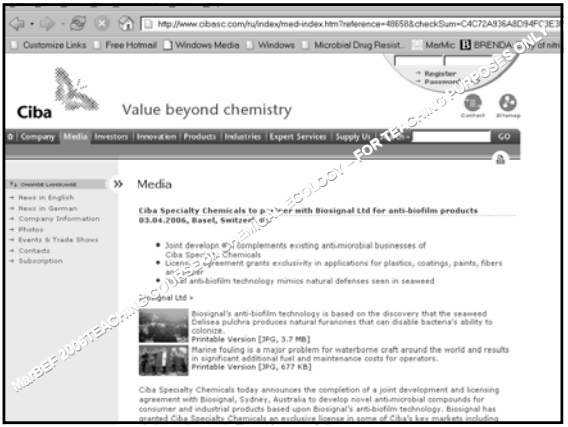
eukaryotic usage of prokaryotic signal

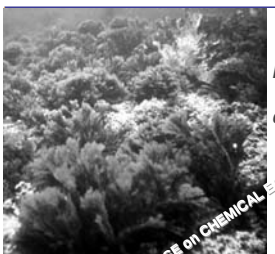
Bacterial biofilm =>
settlement of Eukarya
⇔Biofouling
⇔Economic loss

⇔Transport of organisms with ships
⇔invading species
⇔habitat protection, e.g. Australia


Arriving in Australia with a clean hull
New rules for vessels entering Australia
AQIS fact sheet
New biofouling protocols
The Australian Quarantine and Inspection Service (AQIS) is preparing to introduce the world's first biofouling protocols as part of a national project to keep marine pests out of Australian waters.
From 1 October 2005, a phase in period of voluntary inspections will commence. After a review of the voluntary phase, the requirements will become mandatory. This is expected to occur after 1 October 2006.
What is biofouling?
Biofouling refers to marine organisms that attach themselves to objects immersed in salt water,
What you need to do
Arrive clean
If your vessel arrives clean in Australia with acceptable documentation, then you will fulfil Australia's biofouling requirements.
Before you leave your last port for Australia:
1. Keep all ancillary gear and internal seawater systems clean of marine pests and growths; and
2. Clean your vessel's hull within one month before arrival; or
3. Apply antifouling paint within one year before arrival; or
4. Book your vessel in to be slipped and cleaned within one week of arrival.

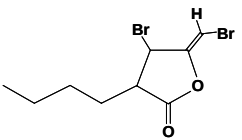

Mozilla Firefox
Tools Help
//www.seanine.com/coating/efficacy/index.html
Windows Media
Windows
Microbial Drug Resist...
MarMic
BRENDA Entry of info...
Product
Efficacy
marine algae
barnacles
raft exposures
Fouling occurs from bacteria, diatoms and protozoa resulting in a slime layer. A slime layer of 1mm can cause an 80% increase in friction and a 15% loss in speed compared to a freshly coated surface.
Formulating a Coating with SEA-NINE

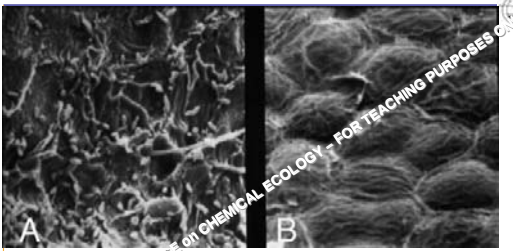




Delisea pulchra
- a red algae producing quorum sensing inhibitors

surface concentration:
100's g halogenated furanones per cm²

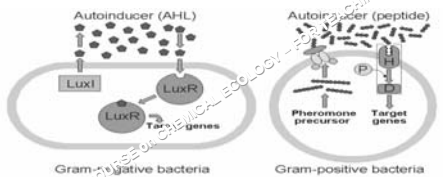


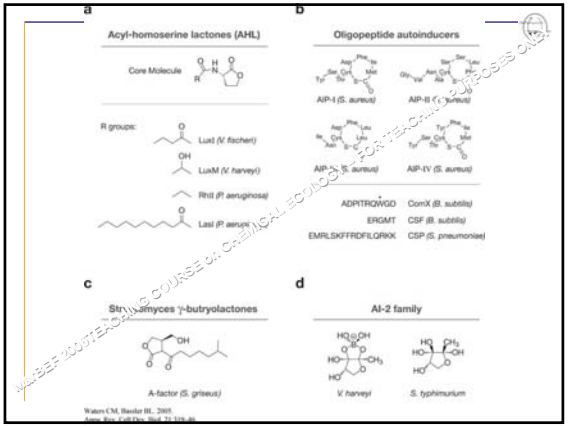


Scanning electron micrograph showing the bacteria associated with the surface of Delisea pulchra (B) and Sargassum sp. (A) a co-occurring brown alga that does not produce the bacterial signal antagonists.

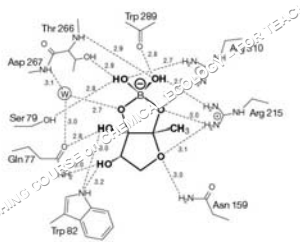
http://www.hermonslade.org.au/projects/HSF_05_9/hsf_05_9.htm

Overview - how cells coordinate behavior





LuxS is “quasiuniversal”



Examples of bacteria that use acyl-homoserine lactones

Bacteria	Function
Vibrio fischeri	luminescence
Aeromonas hydrophila	proteases
Agrobacterium tumefaciens	conjugation
Burkholderia cepacia	siderophores
Chromobacterium violaceum	antibiotics
Erwinia chrysanthemi	pectinase
Pseudomonas aeren?ciens	phenazines
Pseudomonas aeruginosa	biofilms, etc
Rhizobium etli	number of nodules
Yersinia pseudotuberculosis	agreggation and motility

High density and high diversity of prokaryotes

Challenge and chance for chemical ecology

Focus on functional aspects

1. principles of cultivation and isolation
2. chemical communication between bacteria
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Bioassays for quorum sensing inhibitors

Strain characteristics	AHL
wild type luminous bacteria (LB)	endogeneous
LB mutant / GMO mutant without AHL production	exogeneous addition
GMO with AHL sensor and with cell-toxic reporter gene	exogeneous addition

Bioassays for quorum sensing inhibitors with luminous bacteria (LB): aiming at loss of light

luminescence requires molecular oxygen as cosubstrate
- \+ on plates
- \+ in shaking liquid dilutions
- +/- in microtiter plates

test of strains: striking on plates
- \+ easy handling
- \- space consuming
- \- test strains may utilize substrate for LB
- \- test strains may harm LB (false positives)

Test of cell-free extracts
- \- solutions may harm LB (false positive)

Bioassays for quorum sensing inhibitors: a positive selection for lack of cell death

Construct of a AHL-dependent promotor and a cell-toxic gene, e.g.

phlA cell death in the presence of phl

sacB cell lysis in the presence of sucrose

⇒ under selective conditions only cells with a suppressed quorum sensing system are viable, are able to grow

Hosts: *Pseudomonas aeruginosa* ⇔ las system
Escherichia coli ⇔ lux system of *V. Fischeri*

T.B.Rasmussen et al. J. Bacteriol. 187, 1799 (2005)

Quorum sensing inhibition

Targeting the AHL biosynthesis

difficult, inhibitors interfere with central cell metabolism

Inactivation of AHLs

pH > 7 => reversible lactone lysis ⇔ reversible inactivation
enzymatic lactonolysis by e.g. AiiA of Gram-positive *Bacillus*
oxidative transformation by enzymes or hypobromous and hypochlorous acid (HOBr and HOCl)
degradation by amino acylase which cleaves the peptide bond

Interfering the reception

blocking the receptor for AHL-binding
enhanced protein degradation of the receptor

Quorum sensing inhibition

Targeting the AHL biosynthesis

- difficult, inhibitors interfere with central cell metabolism

Inactivation of AHLs

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Interfering the reception

- blocking the receptor for AHL-binding
- enhanced protein degradation of the receptor

Latest news:
garlic as source of quorum sensing inhibitors

Garlic extract: blend 150 g of garlic with 300 ml toluene, filtrate, extract the toluene phase with 150 ml water

BALB/c mice with a Th-2 dominated immune response (resembling cystic fibrosis)

daily single subcutaneous injection of 300 µl garlic extract (1.5 % of body weight)

lung infection with 1 x 10^7 cfu *Pseudomonas aeruginosa* in alginate beads

in vivo clearance within 5 days (control still had 1 x 10^5 cfu)

QS signals (QS-controlled genes) of *Pseudomonas aeruginosa*
- support the biofilm formation
- block the activation of polymorphonuclear leukocytes, a key player of the immune defense.

Current model: 50 bulbs of garlic per day for an 80 kg person

Microbiology 151, 3873 (2005)