Coming up in next week's Science:

***E. coli* colonies can "associate" higher temperatures (e.g. human mouth) with impending lack of oxygen (e.g. human gut).**

When exposed to higher temperatures, they alter their metabolism in anticipation of lowering oxygen levels.

"Anticipatory behavior", like Pavlovian conditioning?

Tagkopoulos, Liu, and Tavazolie. 2008. Science (online May 8th)
We question whether homeostasis alone adequately explains microbial responses to environmental stimuli, and explore the capacity of intra-cellular networks for predictive behavior in a fashion similar to metazoan nervous systems. We show that *in silico* biochemical networks, evolving randomly under precisely defined complex habitats, capture the dynamical, multi-dimensional structure of diverse environments by forming internal models that allow prediction of environmental change. We provide evidence for such **anticipatory behavior** by revealing striking correlations of *Escherichia coli* transcriptional responses to temperature and oxygen perturbations—precisely mirroring the co-variation of these parameters upon transitions between the outside world and the mammalian gastrointestinal-tract. We further show that these internal correlations reflect a true associative learning paradigm, since they show rapid de-coupling upon exposure to novel environments.

Biofilms

I. History

II. Definition

III. Description
   A. General characteristics
   B. Multicellularity
   C. Communication

IV. Variations in structures

V. Biofilms in human disease

History:

-Henrici (1933) – first described that bacteria associate with surfaces

-Zobell, 1945 – marine bacteria colonize glass

-Costerton (1970's)

-rumen bacteria attached to cellulose looked different from those in rumen fluid

-*E. coli* causing scours are "detached" from epithelium of intestine till stained with ruthenium red

-alpine streams carry 8-20 cells per mL, but surfaces of rocks in alpine streams have > 100 million bacteria per cm^2

Implication: planktonic cells are unusual and biofilms are the vast majority of bacterial communities

Bacteria in liquid culture = "planktonic"
-used to study most microbial phenomena prior to 1990's
-used to describe quorum sensing

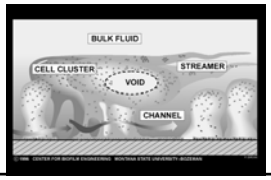
Bacterial climax communities are "biofilms"
-communities of microbes associated with a surface, typically encased in extracellular matrix

-liquid/solid interface
-air/water interface
-no obvious interface (suspended aggregates)

Biofilms are the "norm" and planktonic cells the exception in nature.

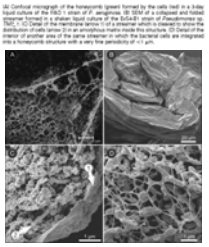
Biofilm gene expression differs 70% from planktonic cells.

Whoops, we've been studying the wrong thing all these years!



Biofilms are **viscoelastic**: deform under shear force; oscillate under high shear force; lose surface attachment when shear exceeds tensile strength.

At high shears biofilms commonly form filamentous streamers which are attached to the solid surface by an upstream "head" while the "tail" is free to oscillate in the flow.



Schaudinn et al., 2007. Microbe 2(5): 231

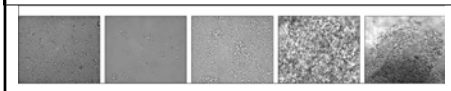
Movies:

Biofilms, streamers, sheer force, and the Sonicare Toothbrush

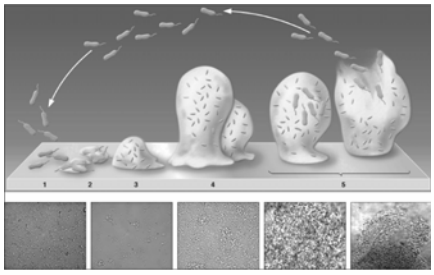
http://www.erc.montana.edu/Res-Lib99-SW/Movies/2002/02-M002.htm

http://www.erc.montana.edu/Res-Lib99-SW/Movies/2002/02-M010.gif

1. Attachment
2. Aggregation and growth into microcolonies
   -mediated by HSLs
3. Maturation of biofilm
   -biomass and thickness governed by AI-2
   -rhamnolipid surfactants maintain water channels
4. Maintenance of biofilm
   -biofilms may "pump" water by changing ionic strength of milieu
   -dispersal
   -programmed cell death



Sauer et al., 2007. Microbe 2(7): 347



The biofilm developmental process in stages. (i) reversible attachment, (ii) irreversible attachment, (iii) maturation-1, (iv) maturation-2, and (v) dispersion.

-organized into microcolonies
-towers and mushrooms
-structural variation among species and between mixed or single-species biofilms
-intervening open-water channels
-oxygen extremely limiting below surface of microcolonies
-gradients of all nutrients, decreasing away from surface

Sauer et al., 2007. Microbe 2(7): 347

Movies:

Water movement through mixed-species biofilm structures as tracked by fluorescent beads

http://www.erc.montana.edu/Res-Lib99-SW/Movies/1995_2000/95-M001_00-M001.htm

What is a "biofilm cell" vs. planktonic cell?

Differential gene expression in *P. aeruginosa, E. coli, V. cholerae, S. pneumoniae, S. aureus,* and *B. subtilis*.

Depends upon state of planktonic cells (dense cultures in chemostat will be doing QS, biofilm likely to do this too)

Depends upon age of biofilm (1d? 5d?)

Depends on method (IVET, microarray, proteome analysis)

Results vary from 1% of genome to 70% of genome being differentially expressed between these states.

Take home message:

Just like in this room, cells sampled represent an **average** of population, and represent various stages of maturity, stress, growth, motility, etc. There's a **range of phenotypic switches over time.**

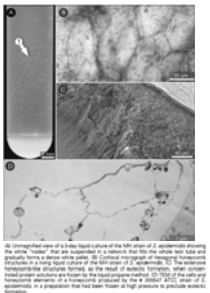
Regulation of normal biofilm formation

Various of these genes required, depending on species… no "core regulator" common to all species for biofilm formation has been identified.

Chemotaxis genes
Flagellar genes
Alginate genes
Sigma factors (RpoN, RpoS)
Membrane transport proteins
Membrane sensor proteins (GacA/S)
Quorum sensing genes (LasR, RhlR)
Signal genes (cyclic di-GMP)

The genes for biofilm formation are not the same as those that stimulate fruiting body/spore formation – the latter tend to be sigma-factor driven (stress/stationary phase).

Biofilm & microcolony structure



A. Aggregates form in liquid cultures of many species after 2-3 d

B. Confocal microscopy reveals honeycombing

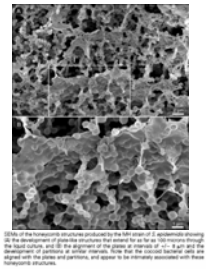
C. Similar structures are formed by freezing dense solutions of proteins

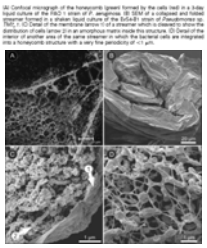
D. SEM, TEM show honeycombing, too… not an artefact?

E. Occur in *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and other spp.

F. Function unknown – structural support?

-no DNA in the matrix !





SEMs of honeycomb structures formed by S. epidermidis: regular, occupied or empty,

Schaudinn et al., 2007. Microbe 2(5): 231

Biofilm characteristics

-multiple cells
-single-species biofilms are rare in nature

-extracellular matrix present (composition varies; polysaccharides common)

-complex architecture/physical heterogeneity – shapes vary
-chemical heterogeneity: physiochemical gradients exist

-cell density and species composition change over time (recruitment/shedding)
Biofilm towers and mushrooms "dissolve" under nutrient limiting conditions, with sessile cells reverting to planktonic phenotype *(is there a "detachment signal" like A-factor in Myxococcus?)*

-communication is essential: biofilm formation frequently shown to require homoserine lactone or other signals
in *P. aeruginosa* biofilms, *lasI* induced first in all cells, then *rhlI* induced later in a subset of cells. Division of labor induced by signaling?

Movies:

Diffusion of small molecule (rhodamine) into a biofilm: gradients

http://www.erc.montana.edu/Res-Lib99-SW/Movies/2005/05-M001.htm

Seething and detachment of cells in center of microcolonies, driven by nutrient starvation

http://www.erc.montana.edu/Res-Lib99-SW/Movies/2004/04-M003-4.htm

Biofilm characteristics

Cells are evenly spaced, further apart than explained by matrix extrusion around cells and their neighbors; optimum for nutrient exchange (*pili connecting/pushing apart sessile cells?)*

Horizontal gene transfer rates are orders of magnitude higher than in planktonic cultures (*F pili connecting sessile cells?)*

Biofilm structure varies with nutrient source*:* in 2-species flow-cell, mixed colonies if *Pseudomonas* could not metabolize supplied nutrient but could utilize *Burkholderia* by-product, but single species microcolonies when both spp could utilize supplied nutrients

Division of labor includes sacrifice: in *B. subtilis* biofilms, spores tend to form at tips of aerial structures at air-exposed surface. Mother cells lyse to release spore. In *Myxococcus*, stem cells sacrifice selves to spore. In *Streptomyces*, substrate mycelium sacrifices cells to spore.

Are biofilms "multicellular" entities?

Multicellularity: the state of being composed of many cells

-cells communicate
-cells coordinate activities for the good of the group
-individual cells make investments for the good of the group
-some cells sacrifice their ability to reproduce " "
-groups of cells are the unit of selection, not individuals
-or… genome is level of selection?

Daughter cells of mitosis (us) or binary fission (microbes): r = 1; remember Hamilton's rule!

Extreme examples of "altruism" explained by multicellularity:

-stem cells of *Myxococcus* fruiting body
-heterocyst (N_2 fixing cell) at terminus of cyanobacterial filament; cannot reproduce
-substrate mycelium in *Streptomyces*
-apoptosis in biofilms

***Myxococcus* multicellular behavior**

Coordinated movement, unlike random walk of chemotaxis
-slime layer contains fibrils
-slime and fibrils are "wrapped" around all cells
-pilus of one cell anchors on fibril of another
-retraction of pilus = "pulling"
-called "S motility", named for slime trails

Five signals necessary for fruiting body formation
-A signal: mix of 6 **amino acids** at low concentration
-C factor: for "**contact**" signal - membrane-bound proteins at cell poles
-B, D, and E: remain unknown, but mutants can be restored to normal fruiting body formation by extracellular complementation
-guide group from one developmental stage to next
-A starts signaling cascade, then C produced. C autoinduces till enough cells are swarming to form fruiting bodies.

Cyanobacterial multicellular behavior

Under conditions of limiting N, cyanobacteria can fix N_2.

Problem:

-fixing N_2 is energetically expensive
-ATP supplied by photosynthesis
-photosynthesis generates O_2
-O_2 poisons nitrogenase

Solution: division of labor

-10% of cells become heterocysts
-heterocysts protect nitrogenase from oxygen
-vegetative cells provide heterocysts with photosynthate
-heterocysts provide vegetative cells with fixed N

-heterocysts secrete small peptide that inhibits differentiation of other heterocyst cells nearby

***Streptomyces* multicellular behavior**

Streptomyces forms aerial hyphae and exospores.

Division of labor:

-"substrate mycelium" of highly branched, densely packed hyphae dig into substratum and take up nutrients
-some hyphae secrete surfactants, permitting escape from substratum and aerial growth
-substrate mycelium secretes antibiotics and obtains nutrients
-substrate mycelium lyses and "feeds" aerial hyphae
-aerial hyphae produce exospores by multiple cell divisions

Exospore formation is regulated by signaling:

-four small diffusible signals coordinate timing of antibiotic production by substrate mycelium
-six signals are required for coordinated formation of aerial mycelium
-extracellular complementation hints at signals but only one known:
  - γ-butyrolactone (controls antibiotic production)
  - oligopeptide (controls aerial mycelium development)

Communication in biofilms

Signals may not reach average concentrations seen in planktonic studies

-Cells close together
-matrix slows diffusion
-critical local concentrations of signals, higher than "average" that we can chemically measure

-development of biofilms likely resembles embryology of higher life forms, controlled by localized signaling by hundreds of signals

Biofilms optimize metabolic processes:

-metabolic cooperation and formation of stable species consortia (reduces diffusion)
-corrosion of metallic surfaces (e.g. rust)
-cell-cell signaling (first studied in planktonic cultures)

Biofilms colonize artificial and biological surfaces:

-Foley catheter from urinary tract
-cardiac pacemakers
-Jarvik artifical heart
-contact lenses
-intrauterine contraceptive devices
-epithelial cells

…not all are pathogenic!

Biofilms are seen in 65 to 80% of all infections treated in the developed world.

Limitations of biofilm observation

Micrographs are snapshots in time; do not portray plasticity of structure, cell movement, etc.

Chemical analyses are "averages" over sample and do not portray hotspots of high concentrations (e.g. signals)

Single-species biofilms are unnatural – in vivo, biofilms comprise from several to hundreds of species

Etc.

Biofilms in disease

I. Reservoirs

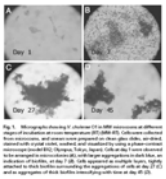
II. Antibiotic resistance

…biofilms that form *in situ*, that is, in the surface water, are more likely to account for seasonal cholera epidemics…

Vibrio cholerae 01 enters dormant state when conditions don't favor growth: small coccoid cells

Autoclaved Bangladesh pond water and inoculated with *V. cholerae*.

Gradually formed biofilms, and culturable curved rods → small coccoid nonculturable cells

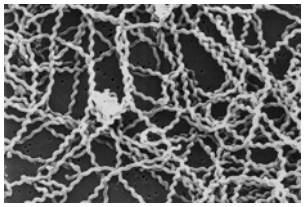


After 495 days, dormant cells from biofilms but not those collected as free cells could be cultured IF passed through animals.

Conclusion: Biofilms help cholera persist between epidemics

Alam et al., 2007. PNAS 104: 17801

Biofilms in leptospirosis



Leptospira interrogans are long, thin motile spirochetes that may be free-living or associated with animal hosts and survive well in fresh water, soil, and mud in tropical areas. (Credit: Janice Carr / CDC)

Leptospira interrogans:

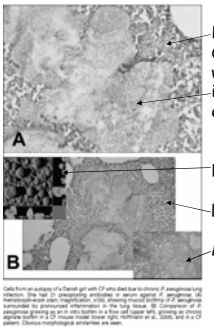
Major health problem in SE Asia, S. America

Causes severe liver damage, meningitis

Up to 20% of cases fatal

Carried in rat kidneys, spread in urine to water sources

Not planktonic, but biofilms, in water

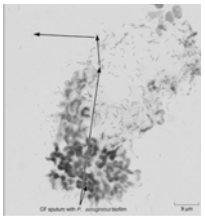


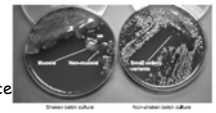
Biofilm in CF lung with inflamed epithelium

Flow cell

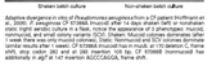
Lung

Mouse





Adaptive divergence



Antibiotic resistance in biofilms

Bacteria in biofilms exhibit different physiology than planktonic cells.

Medical context:
Tolerant to 1000X higher levels of antibiotics, phage, antibodies, and antimicrobial peptides than those required to decimate populations of planktonic cells

-cystic fibrosis patients (children)
-UTI on catheters

Why?

A. EPS limits diffusion or chelates certain compounds

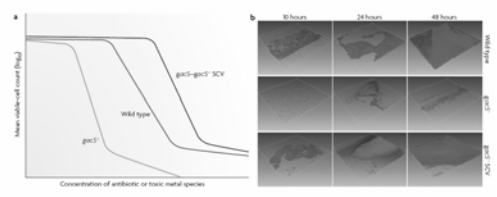
B. Different physiological states = differential resistance (exponential, stationary, dormant)

-Adaptive stress responses make cells more resistant

-Persister cells (dormant = target bound by antibiotic but no effect?)

-Slow growth of cells = tolerance to antibiotic

Phenotypic variation in *Pseudomonas aeruginosa* is linked to biofilm multidrug and multimetal resistance



Harrison et al., 2007. Nature Reviews Microbiology 5: 928

Bacteria in biofilms exhibit different physiology than planktonic cells.

Environmental context - the following processes occur at different rates in the presence of planktonic vs. biofilm cells:

C cycling and nutrient cycling

Chemical reactions in bioreactors

Toxic chemical degradation

Industrially important metabolisms

e.g. oxygen, actively respired by first few cell layers such that a few microns in, oxygen concentrations may be only 40% of the surrounding fluid

e.g. methane by methanogens, or HSL by signaling cells

An intermediate, common in mixed biofilms - e.g. nitrate → ***nitrite*** → N_2, or the benzoate in assigned paper

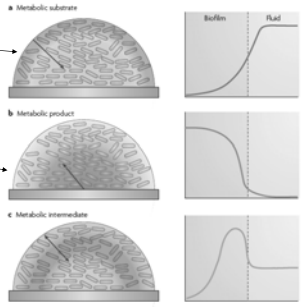


Figure 2 | **Chemical heterogeneity in biofilms.** Three qualitatively distinct patterns of chemical heterogeneity arise in biofilms owing to reaction–diffusion interactions for a metabolic substrate (blue; a), a metabolic product (orange; b) and a metabolic intermediate (green; c). The concentration of a substrate that is consumed in the biofilm decreases with depth into the biofilm and distance away from the source (a). Conversely, a metabolic product is more concentrated inside the biofilm (b). A metabolic intermediate that is both consumed and produced within the biofilm can exhibit

Stewart and Franklin. 2008. Nature Reviews Microbiology 6: 199

Chemical and physiological heterogeneity in biofilms:

Nutrients, O_2

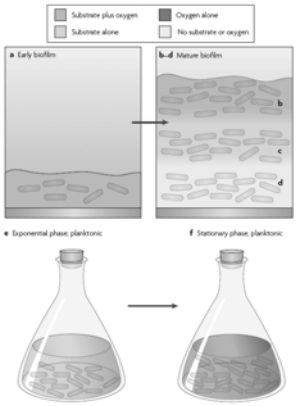


Figure 3 | **Physiological heterogeneity in a single-species biofilm.** A thin biofilm at an early stage of development (a) is replete with both substrate and oxygen. In the mature biofilm, environments that contain both substrate and oxygen (b), substrate but no oxygen (c) and neither substrate nor oxygen (d) can occur. In an exponential-phase planktonic culture, substrate and oxygen are both present (e), whereas in a shaken stationary-phase culture, substrate can be depleted but oxygen will still be present (f).

Stewart and Franklin. 2008. Nature Reviews Microbiology 6: 199

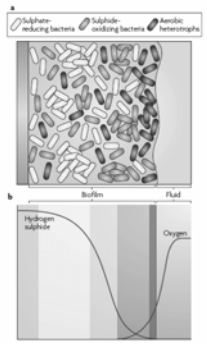
Very different from mature biofilm

Chemical and physiological heterogeneity in biofilms:

Electron donors
Electron acceptors
Microbial metabolic capabilities

Figure 4 | **Physiological heterogeneity in a mixed-species biofilm.** Three groups of microorganisms are distributed within a biofilm (a), and each experiences a range of chemical microenvironments (b). In panel b, shaded zones denote the regions of oxygen respiration (red), sulphide oxidation (blue) and sulphate reduction (yellow), and the black curves describe the concentration profiles for hydrogen sulphide and oxygen.

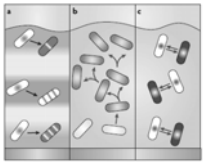
Biofilm exposed to water containing O_2, sulfate, and C sources



Stewart and Franklin. 2008. Nature Reviews Microbiology 6: 199

Genetic heterogeneity: insurance policy by biofilm cells?

Figure 5 | **Multiplicity of phenotypic states in biofilms.** The three hypothesized mechanisms of phenotypic diversification in a biofilm. **a** | Physiological adaptation. Cells respond adaptively to local environmental conditions by turning on or off certain genes; the responses depend on the local chemical microenvironment and, therefore, allow for a range of distinct localized adaptations. **b** | Genotypic variation and natural selection. A mutation or chromosomal rearrangement results in a variant (purple) that multiplies according to its fitness in the biofilm. **c** | Stochastic gene switching. Cells toggle between discrete physiological states by gene switching, which is random in nature.



a. Physiological adaptation
b. Evolutionary/adaptive adaptation
c. Variable gene expression by neighboring cells

Stewart and Franklin. 2008. Nature Reviews Microbiology 6: 199

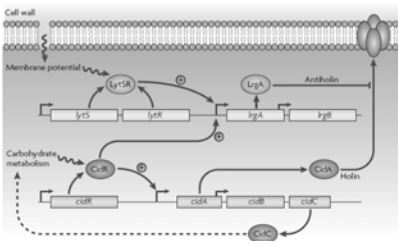


Figure 1 | **The cid/lrg regulatory network.** The *Staphylococcus aureus cidA* and *lrgA* genes encode homologous hydrophobic proteins that are believed to function as a holin and antiholin, respectively. The *S. aureus cidB* and *lrgB* genes also encode homologous hydrophobic proteins[37], but the functions of these proteins are unknown. It is proposed that the LytSR two-component regulatory system senses decreases in membrane potential and responds by inducing *lrgAB* transcription. The CidR protein, a LysR-type transcription regulator, enhances *cidABC*, *lrgAB* and *alsSD* (*alsSD* is not shown) in response to carbohydrate metabolism[38,39]. The *cidC* gene encodes pyruvate oxidase[38].

Bayles, K.W. 2007. Nature Reviews Microbiology 5: 721

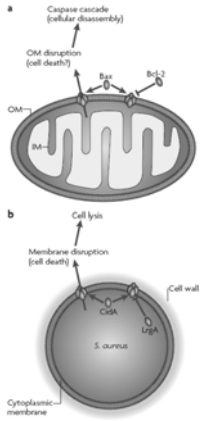


Figure 2 | **Common strategies that control the onset of cell death. a** | The interactions between the eukaryotic proteins Bax and Bcl-2 within the mitochondria are central to the control of apoptosis. Bax oligomerization within the outer mitochondrial membrane (OM) leads to the disruption of this membrane, the release of proteins (cytochrome *c*) that trigger the caspase cascade within the cytoplasm and cellular disassembly. The homologous Bcl-2 protein inhibits Bax oligomerization and, thus, prevents apoptosis. **b** | The *Staphylococcus aureus* proteins CidA and LrgA are believed to function as a holin and antiholin, respectively. Holins oligomerize within the bacterial cytoplasmic membrane causing cell death and, by virtue of their effects on murein hydrolase activity, cell lysis. Antiholins are homologous proteins that inhibit holin oligomerization and, thus, prevent bacterial death and lysis. IM, inner mitochondrial membrane.

Bayles, K.W. 2007. Nature Reviews Microbiology 5: 721

Why cell death?

-release DNA, which has role in biofilm stability
-eliminate damaged individuals
-free up nutrients
-maintain space

Starvation-induced dispersion in *Pseudomonas putida* biofilms

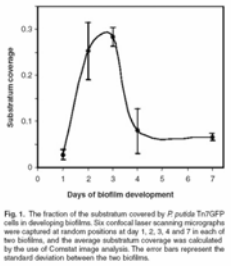


Fig. 1. The fraction of the substratum covered by *P. putida* Tn7GFP cells in developing biofilms. Six confocal laser scanning micrographs were captured at random positions at day 1, 2, 3, 4 and 7 in each of two biofilms, and the average substratum coverage was calculated by the use of Comstat image analysis. The error bars represent the standard deviation between the two biofilms.

Gjermansen et al., 2005. Environmental Microbiology 7: 894

Starvation-induced dispersion in *Pseudomonas putida* biofilms

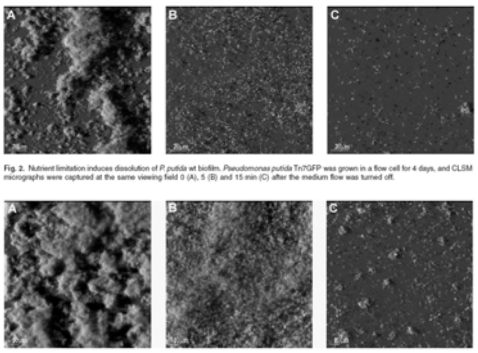
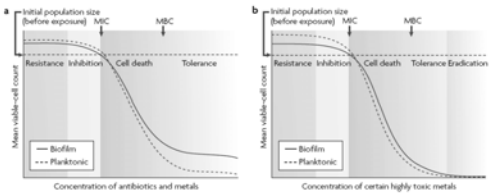


Fig. 2. Nutrient limitation induces dissolution of *P. putida* wt biofilm. *Pseudomonas putida* Tn7GFP was grown in a flow cell for 4 days, and CLSM micrographs were captured at the same viewing field 0 (A), 5 (B) and 15 min (C) after the medium flow was turned off.

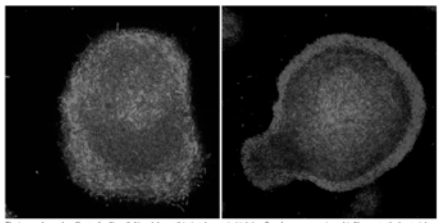
Fig. 3. Nutrient limitation induces dissolution of *P. putida* tbM biofilm. *Pseudomonas putida* tbM was grown in a flow cell for 4 days, and CLSM micrographs were captured at the same viewing field 0 (A), 5 (B) and 15 min (C) after the medium flow was turned off.

Gjermansen et al., 2005. Environmental Microbiology 7: 894

Time- and dose-dependent killing of biofilms by metals.



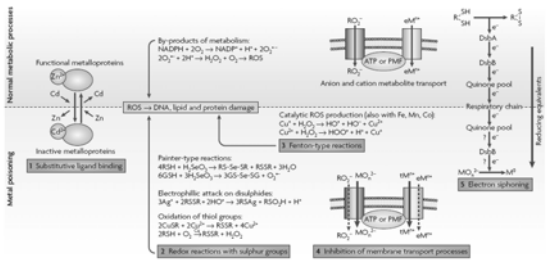
Harrison et al., 2007. Nature Reviews Microbiology 5: 928



The image shows the effects of gallium (left) and the antibiotic tobramycin (right) on *Pseudomonas aeruginosa* biofilms; note the bacterial killing in the interior of the biofilm with the gallium treatment. Image kindly provided by Pradeep Singh.

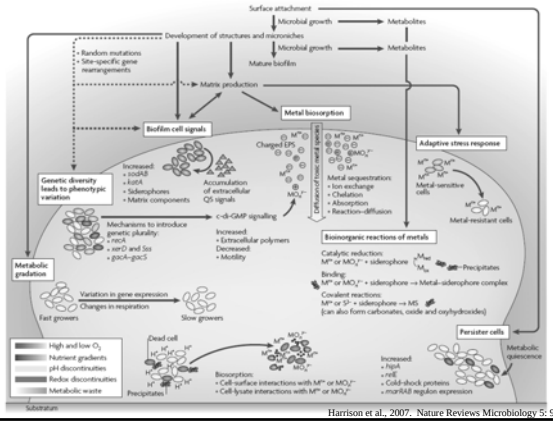
ORIGINAL RESEARCH PAPER Kaneko, Y. et al. The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. J. Clin. Invest. 15 March 2007 (doi: 10.1172/JCI29783)

Biochemical mechanisms of microbiological metal toxicity



Harrison et al., 2007. Nature Reviews Microbiology 5: 928

A multifactorial model of multimetal resistance and tolerance in biofilms



Harrison et al., 2007. Nature Reviews Microbiology 5: 928

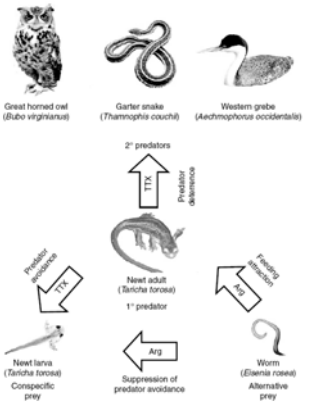
Do same considerations apply to bacteria as other multicellular organisms with multiple life stages?

Selection pressures are different at larval stage than adult stage.

Gene expression differs between larval and adult stages

Silencing of genes or interrupting chemical signaling during development affects organism's function later

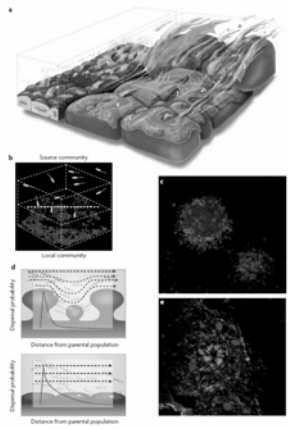
Chemoreception has independent, stage-specific selection pressures leading to changes in physiological properties and behavioral expression.



Schematic depiction of predator–prey interactions involving *Taricha torosa*, and the chemosensory cues that mediate them. Arg, arginine; TTX, tetrodotoxin.

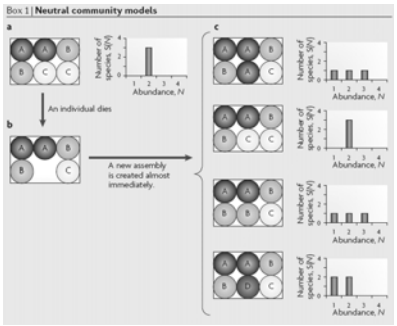
Ferrer and Kimmer, 2007. J. Exp. Biol. 210: 1776

Discussion papers



Biofilms as microbial landscapes

Battin et al., 2007. Nature Reviews Microbiology 5: 76



Battin et al., 2007. Nature Reviews Microbiology 5: 76

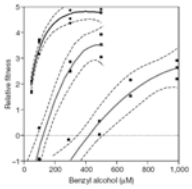


Figure 1 | Coexistence of *P. putida* KT2440 and *Acinetobacter* sp. C6 in structured (biofilm flow chamber) and unstructured (chemostat) environments with benzyl alcohol as the sole carbon source. Fitness measures were determined over 24 h at different concentrations of benzyl alcohol and are the difference between the malthusian parameter of the initially rare species (*P. putida*) and that of the common species (*Acinetobacter*); a fitness of zero indicates equal competitive ability[29]. Lines are second-order polynomials plus 95% confidence curves fitted to the data (a minimum of three replicate measurements were determined at a minimum of three different concentrations of benzyl alcohol in each experiment). The red line denotes the fitness of ancestral *P. putida* in chemostat culture, the blue line the fitness of *P. putida* in biofilm flow chambers, and the black line the fitness of the derived rough variant of *P. putida* in biofilm flow chambers. The threshold concentration of benzyl alcohol above which *P. putida* and *Acinetobacter* coexist is indicated at the point at which each curve crosses the reference line. A curve for the fitness of the derived rough variant of *P. putida* mutant in chemostat culture is not shown, but in this environment this genotype was unable to increase in frequency against *Acinetobacter* populations at benzyl alcohol concentrations lower than about 950 μM (at 1 mM benzyl alcohol the fitness of derived *P. putida* was 0.69 (95% confidence interval 0.165–1.215)).

Hansen et al., 2007. Nature 445: 533

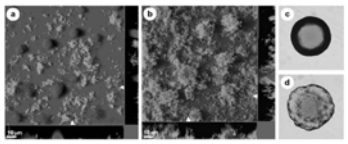


Figure 2 | Characteristic biofilm and colony phenotypes of the ancestral and the derived rough variant of *P. putida*. Flow-chamber biofilms were propagated for 24 h with 500 μM benzyl alcohol as the sole carbon source. **a**, **b**, Confocal scanning laser microscope (CSLM) micrographs were obtained of mixed biofilms containing *Acinetobacter* sp. C6 (red) and ancestral *P. putida* (green) (**a**) and *Acinetobacter* sp. C6 (red) and a rough variant of *P. putida* (green) (**b**). The main panels show simulated fluorescence projection images (SFP): the outer horizontal and vertical panels show a cross-section through the *x*–*z* dimension at the positions marked by the arrows. The images shown are representative of eight images taken from each of two independent flow chambers. **c**, The smooth colony morphology of the ancestral *P. putida*. **d**, The rough colony morphology of a biofilm-derived genotype.

Hansen et al., 2007. Nature 445: 533

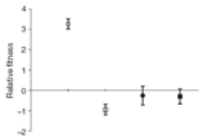


Figure 3 | Competitive fitness of the rough variant of *P. putida*. Fitness was determined relative to ancestral *P. putida* (initial ratio 1:1) in the presence (open symbols) and absence (filled symbols) of *Acinetobacter* in both spatially structured (circles) and unstructured (squares) environments. *P. putida* is unable to grow on benzyl alcohol in the absence of *Acinetobacter*; the fitness of derived *P. putida* in the absence of *Acinetobacter* was therefore determined in environments containing 200 μM benzoate as the sole carbon source. Data are means and 95% confidence intervals; fitness values with confidence intervals that span zero are not significantly different from that of the ancestral genotype. See the text for ANOVA.

Hansen et al., 2007. Nature 445: 533

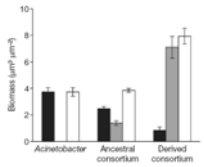


Figure 4 | Productivity of ancestral and derived communities. Biomass was determined after three days by CSLM imaging and COMSTAT analysis. Black bars represent *Acinetobacter*, light grey bars represent *P. putida*, and white bars represent total biomass. Data are means and 95% confidence intervals from three replicate biofilm flow chambers. The negative effect of ancestral *P. putida* on *Acinetobacter* is a consequence of competition for oxygen.

Hansen et al., 2007. Nature 445: 533