Growth and Stress

Microbial growth is limited by environmental constraints

-"food"
-abiotic limits

Microenvironments change dramatically over microscales

Within their limits, individuals and populations of microbes adapt to stresses metabolically and structurally

At limits, stressed microbes invite genetic change at population level

(different from conjugation, transformation, transduction; involves random mutation with a bias)

A little food for thought …

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 (2^8)
0.5	2	4.5	512 (2^9)
1	4	5	1,024 (2^{10})
1.5	8	5.5	2,048 (2^{11})
2	16	6	4,096 (2^{12})
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 (2^{19})

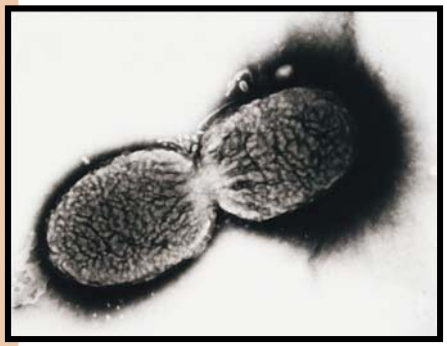


Figure 6-6a Brock Biology of Microorganisms 11/e

-*E. coli* cells, weighing 1 pg (10^{-12}) gram, have a 20 minute doubling time.

-If a single *E. coli* cell underwent unlimited growth and division 48 hours, the resultant offspring would weigh 4000X the weight of the earth!

In stark contrast, deep subsurface microbes are thought to divide only once every 45-300 years

food

Nutrient constraints: *(macro/micronutrients in gut vs. rock)*

Energetic contraints/redox potential: *(fermentation vs. sulfate reduction by radiolytically generated H_2)*

Abiotic constraints:

-water availability *when water is absent, actively metabolizing cells are absent*

-temperature

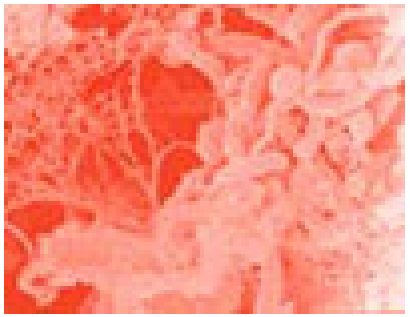
-pressure

-oxygen

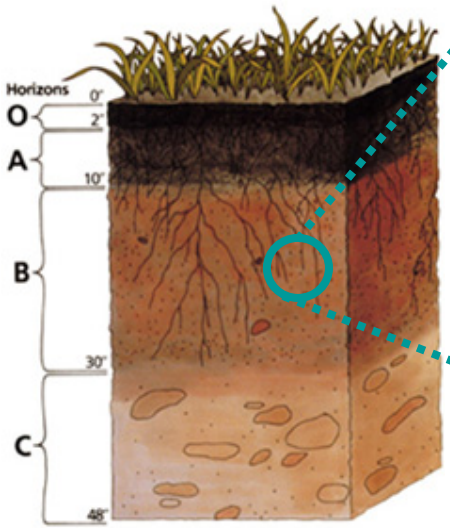
-pH

-salinity

-light



Life in the Soil: feast or famine lifestyle



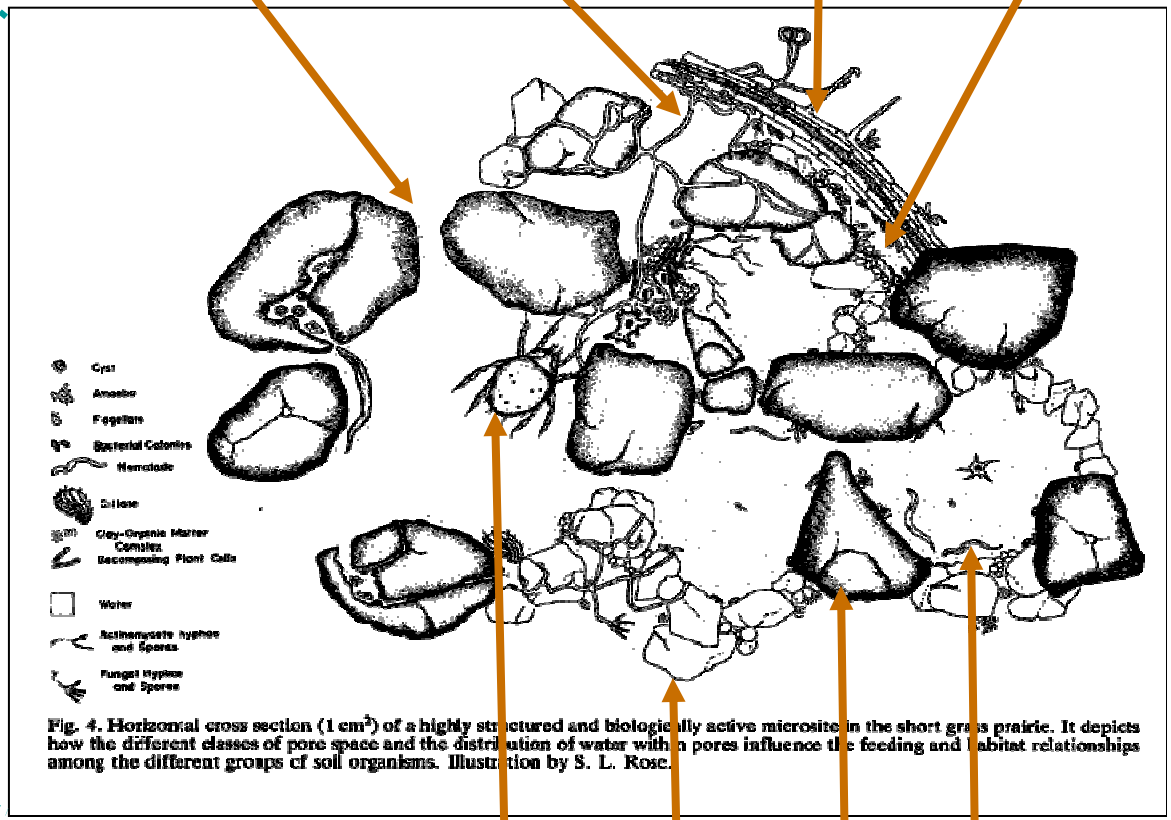
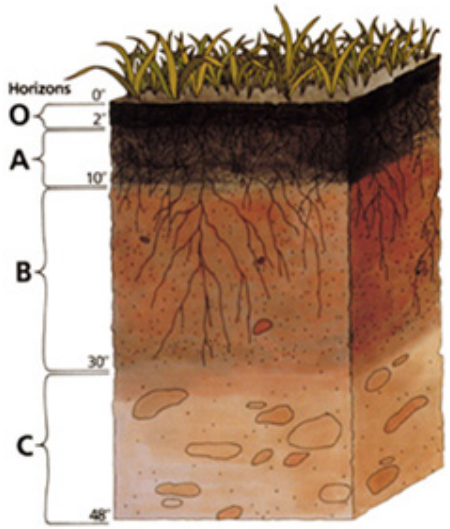


Fig. 4. Horizontal cross section (1 cm²) of a highly structured and biologically active microsite in the short grass prairie. It depicts how the different classes of pore space and the distribution of water within pores influence the feeding and habitat relationships among the different groups of soil organisms. Illustration by S. L. Rose.

http://archive.niees.ac.uk/talks/egenomics_2006/kowalchuk.ppt



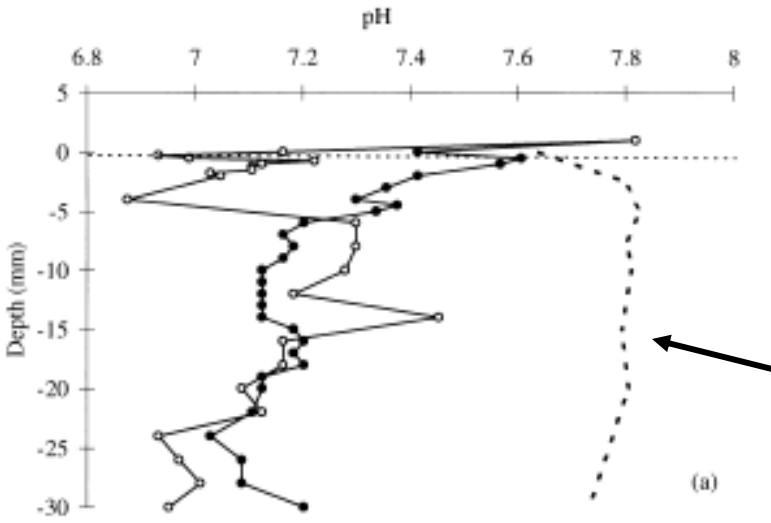
Between microenvironments (μM), extreme changes:

Physical differences –
temperature, moisture, pH, oxygen, clay adsorption…

Biotic changes -
Nutrients depleted during decomposition, waste products accumulate near hotspots of decomposition, antibiotics/toxins secreted

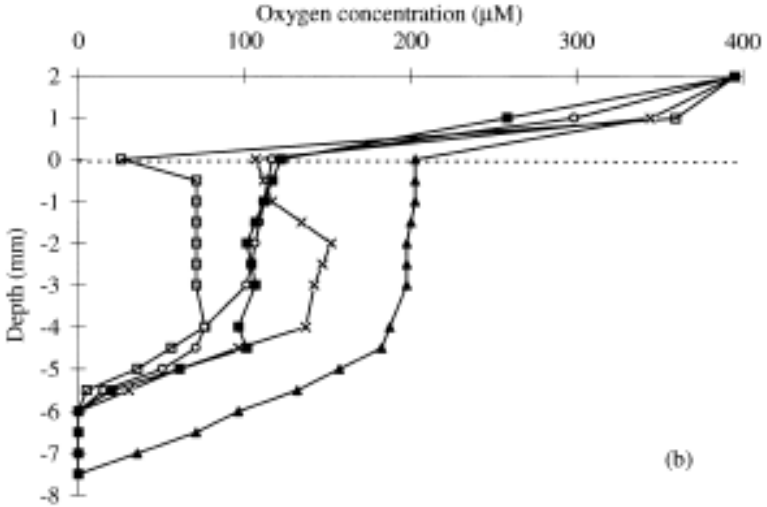
Biodegradable vs. bioavailable:
organic matter may eventually be broken down, but frequently over long periods and by many microbial processes. Not necessarily available (tied up in humic substances or adsorbed to clay).

pH and oxygen levels in river surface sediment



Microelectrode profiles taken from different locations are shown together.

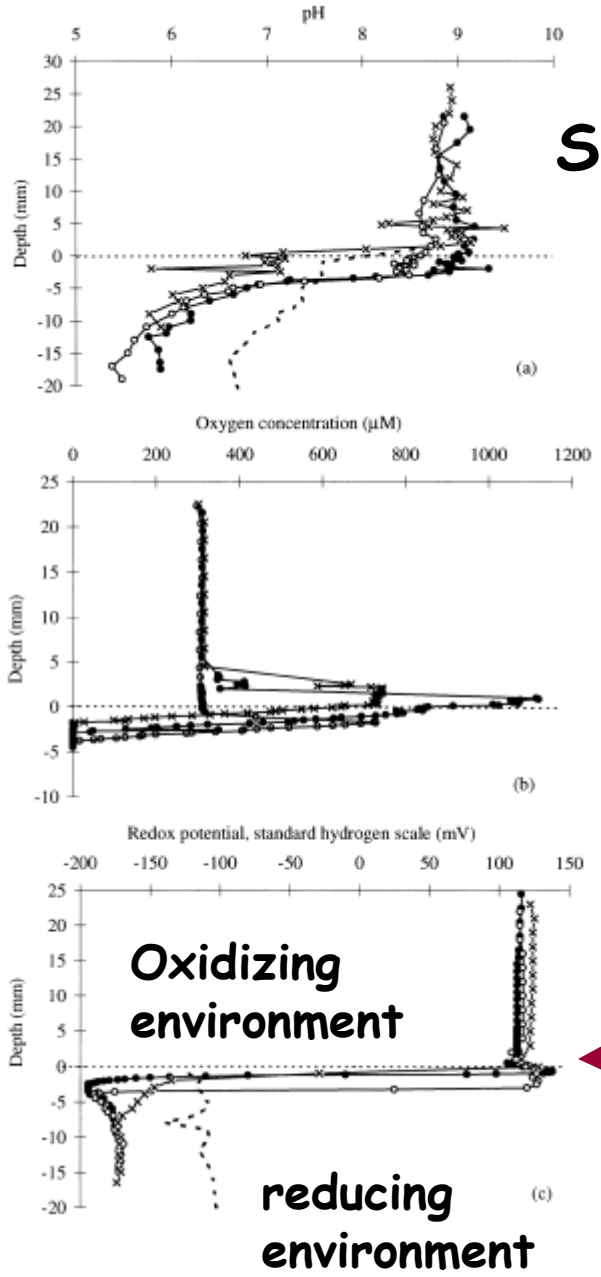
pH of pore water after mechanical sectioning of the sediment (- - -).



Top ~2 mm represent a complex photosynthetic biofilm involving a succession of diatoms, green algae and cyanobacteria.

WINTER

Woodruff et al., 1999. Freshwater Biology 41: 73.

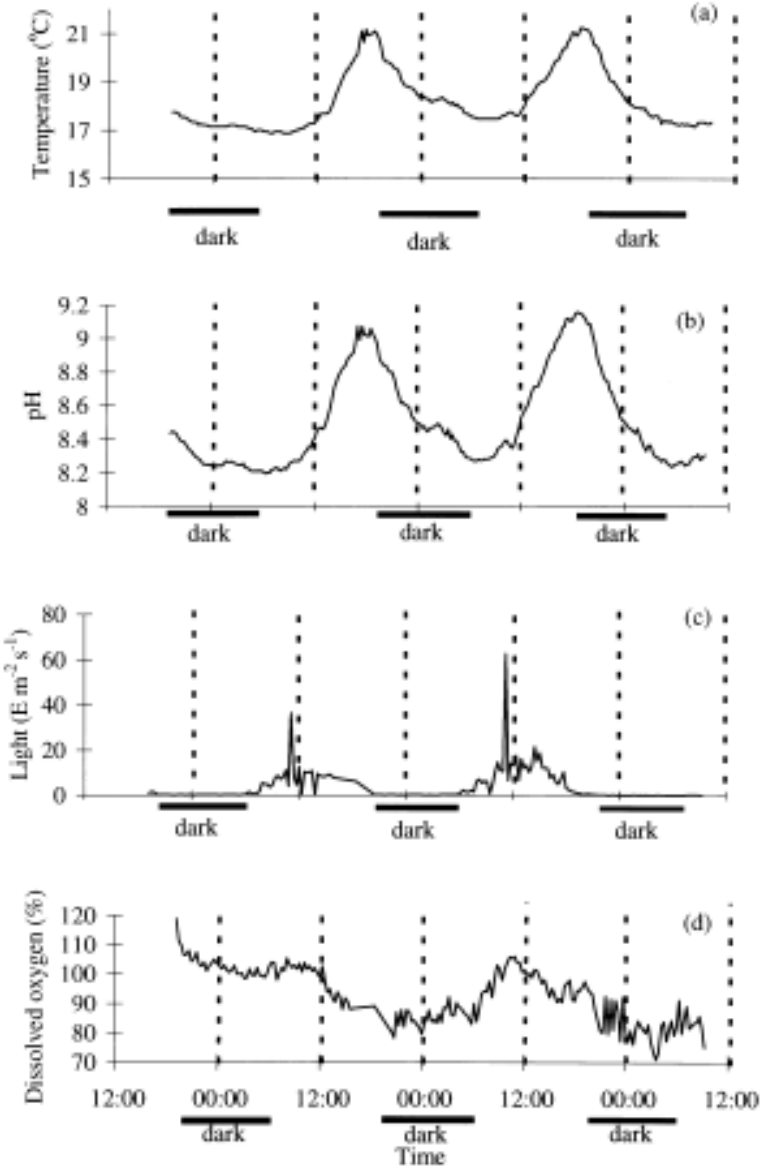


SUMMER – more biomass, similar trend

Why the sudden drop in redox potential?

Woodruff et al., 1999. Freshwater Biology 41: 73.

Measurements of river water taken at 30-min intervals to show diurnal changes



(a) temperature (°C)

(b) pH

(c) light (E m–2 s–1)

(d) dissolved oxygen (% DO)

…light also causes seasonal changes in nutrients, photosynthesis…

…too much UV or PAR may be stressful to unadapted cells

Woodruff et al., 1999. Freshwater Biology 41: 73.

Effects of Temperature on Growth

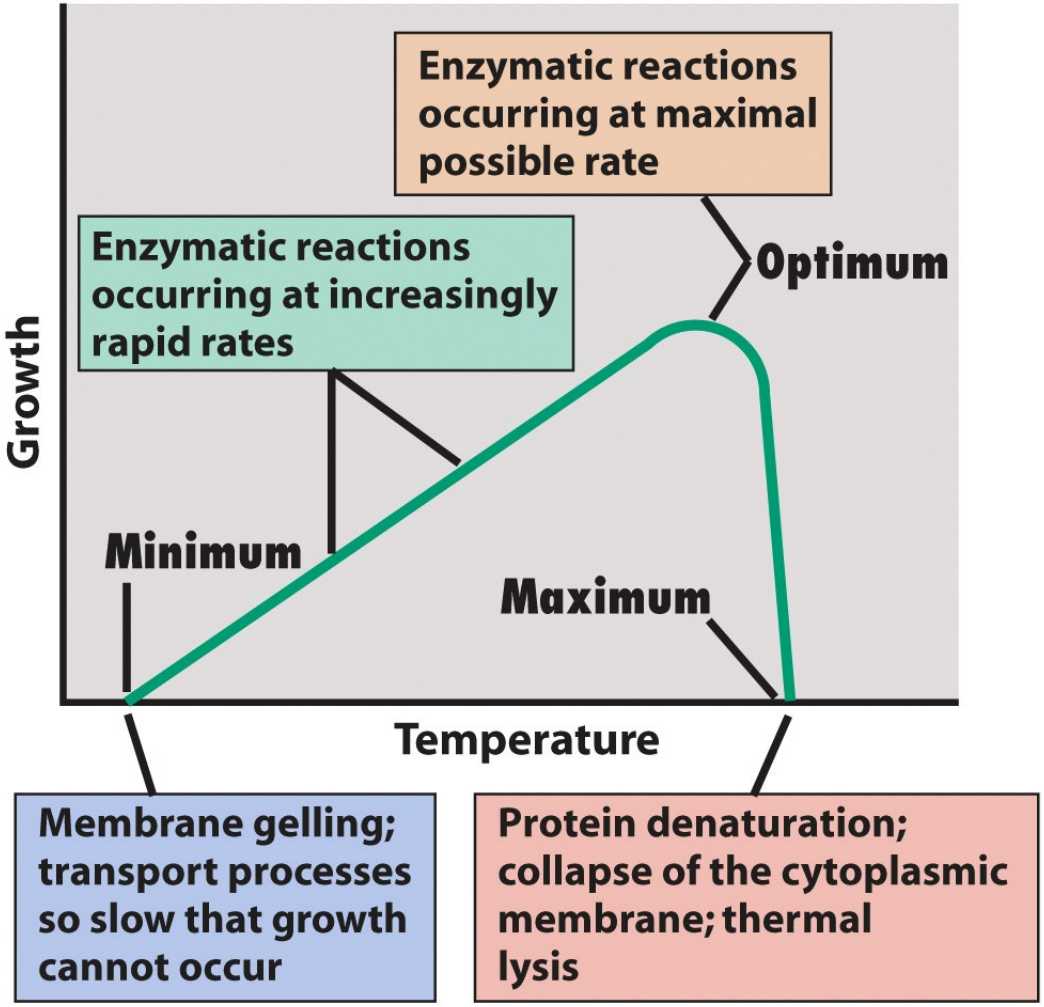


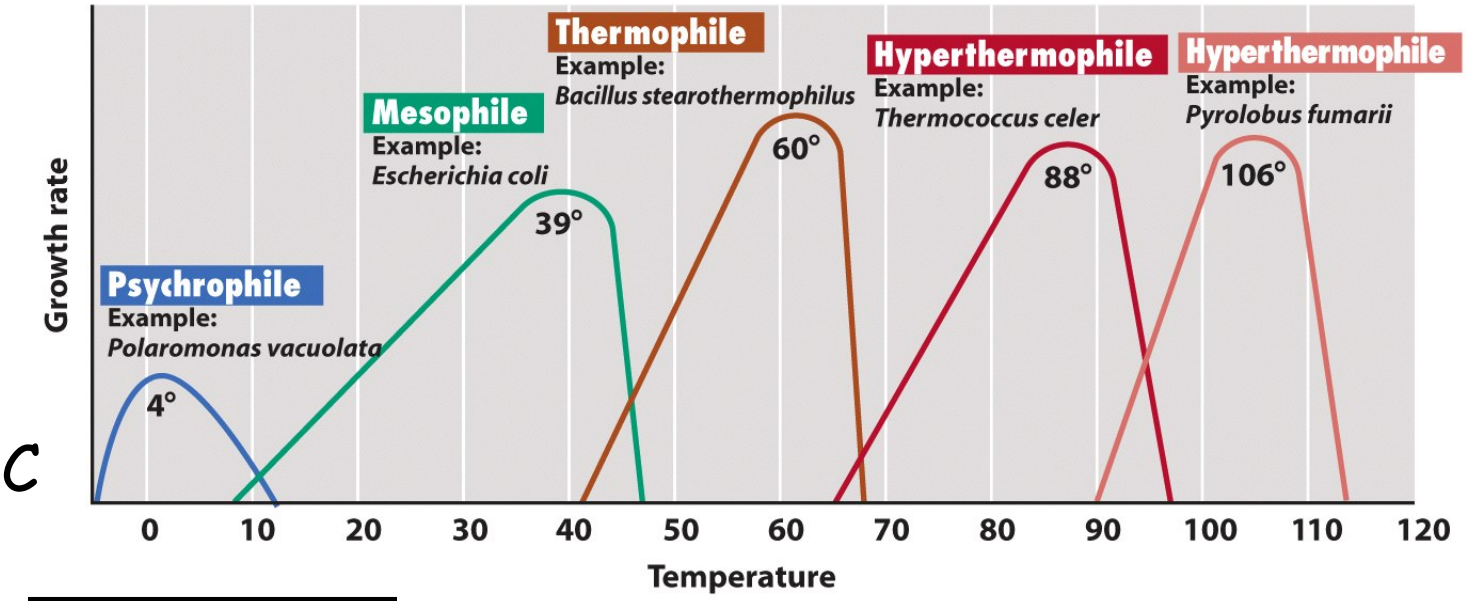
Figure 6-16 Brock Biology of Microorganisms 11/e

- Rule of 10: generally, an increase in temp of 10°C will increase metabolic rate by 2X or 3X.

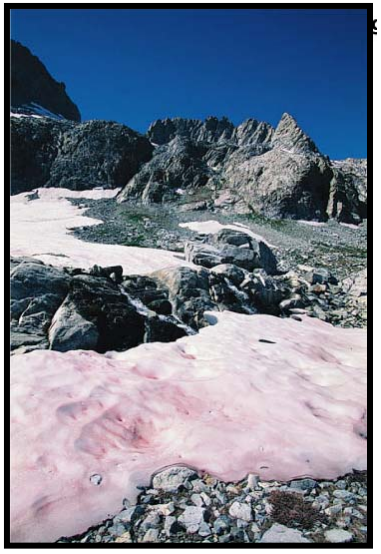
- Limit: when emperature alters 'shape' of enzymes and lipids

- Extremophiles have proteins and membrane lipids with different structural properties than mesophiles

-40°C



121°C



Chlamydomonas nivalis (algae)

http://www.konkle.com/illustration/blacksmoker01.html

pH affects microbial growth

-Organisms are sensitive to changes in acidity because H^+ and OH^- interfere with H bonding in proteins and nucleic acids

-enzymes require a specific pH to function (optimal pH)

Neutrophiles:

Most bacteria and eukaryotes

Acidophiles:

-e.g. many fungi, Archaea
-*Helicobactor pylori,* causal agent of stomach ulcers
    -Grows in stomach: < pH 2

Alkalinophiles:

-e.g. *Bacillus* spp.; Archaea
-*Vibrio cholerae* (causal agent of cholera)
    -Grows best at pH 9 in water (contaminant)

Adaptation

Physiologists' meaning: phenotypic adjustments of organism to adjust to environment

-changes in protein or lipid profiles to adapt to temperature changes

-expression of efflux pumps to resist toxic compounds and heavy metals

-movement (flagellae, gas vacuoles

-attachment/adhesion

-slower growth rates

- miniaturization

-use of endogenous reserves

-regulation of protein turnover

Adaptation

Evolutionary biologists' meaning: natural selection for genetic changes that encode traits increasing fitness in a particular environment.

Acquired by:

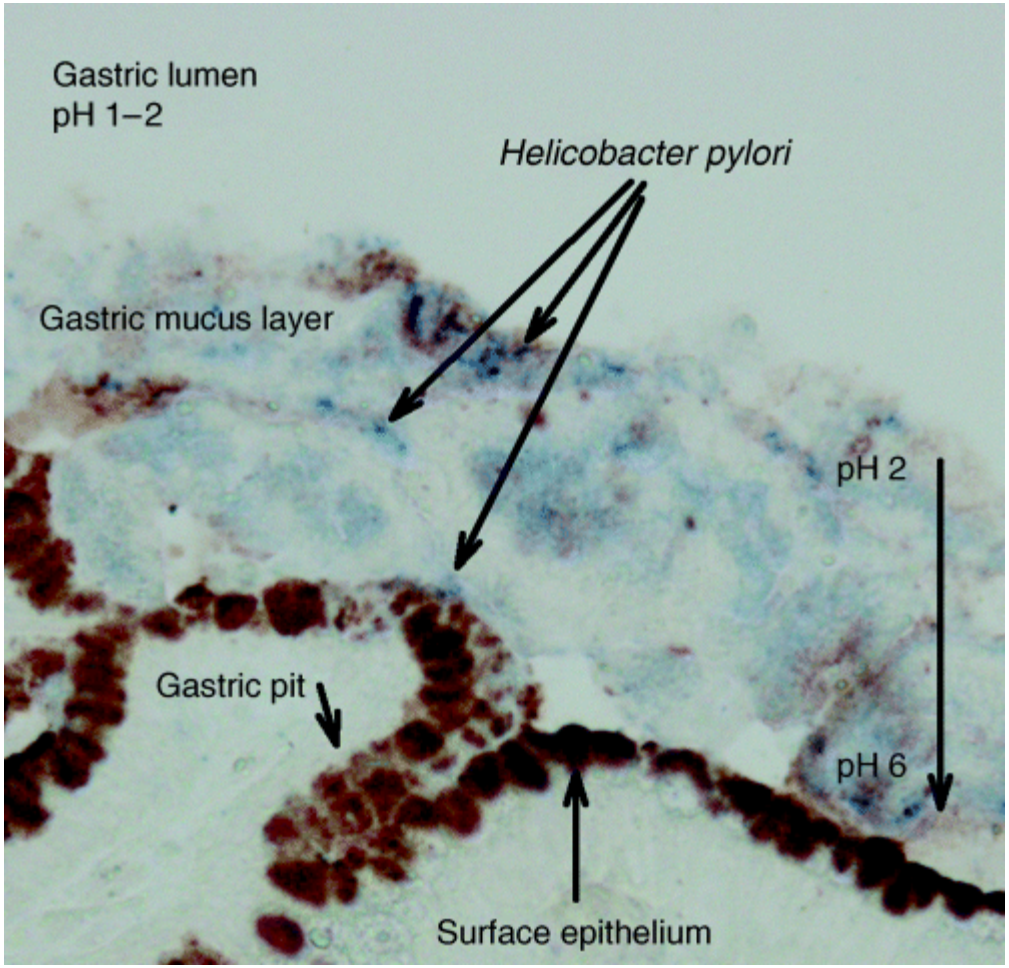
Mutation of existing DNA

Uptake of new DNA (conjugation, transformation, transduction)

Helicobacter pylori on acid:

Polar flagellae aid in pH-driven chemotaxis away from stomach lumen and into mucous lining.

Flagellae are necessary but not sufficient for survival in stomach.



Clyne et al. 2008. FEMS Microbiol. Lett. 268: 135

Helicobacter pylori on acid:

Senses acid pH via two-component regulator, ArsS (histidine kinase) and ArsR (response regulator). ArsSR is necessary but not sufficient for survival in the stomach. ArsR triggers expression of:

Urease (hydrolyzes the limited amounts of urea available in the stomach to generate ammonia and CO_2, which increases the pH)

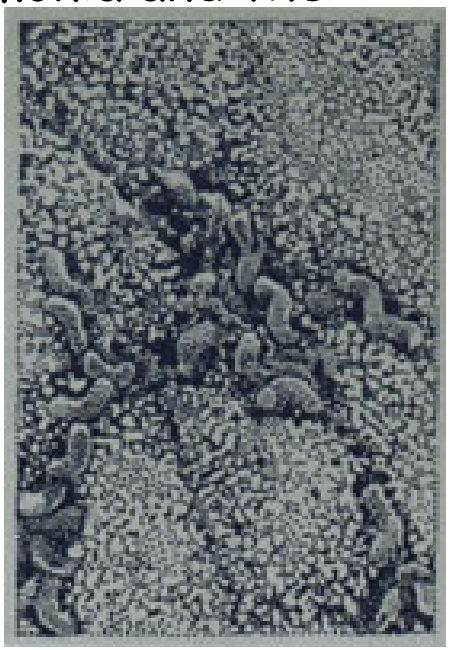
Carbonic anhydrase (converts CO2, produced by urease, to HCO_3)

Amidases (hydrolyze short-chain amides to produce ammonia and the corresponding organic acid)

Downregulation of outer membrane protein production

Formation of biofilms

Other acid tolerance responses



Agrobacterium tumefaciens on acid

"Sensing" of plant wound

Plant wounds have characteristic chemistry: phenolics, sugars released.

Chemotaxis towards sugars, phenolics

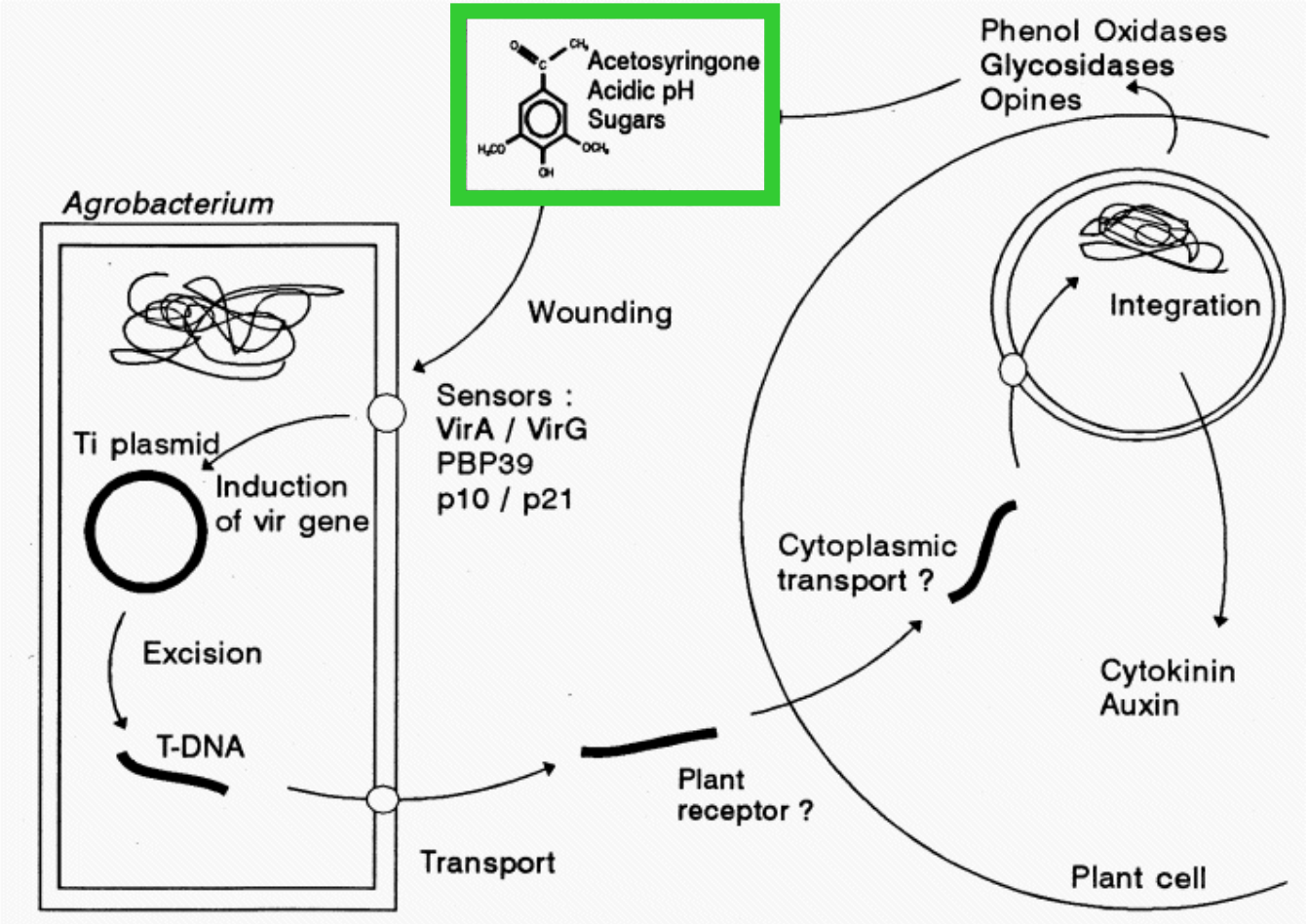
How to tell if "inside" plant?

-pH of apoplast is at least 10-fold lower than soil water (~5.5)

(Sensed by ChvG/I two component system AND by VirA/G "virulence induction" system, also a two-component pair; control overlapping large global regulons)

Certain acid-inducible genes also present in *Rhizobium* and many plant pathogens, function not always clear

How does *Agrobacterium* initiate infection?



Pressure affects microbial growth

Hydrostatic Pressure (special habitats, e.g. pressure of water column)

-pressure increases by 1 atm (0.1 MPa) for every 10 m depth

-most microbes tolerate 1-400 atm steady pressure

-reaction rates for some enzymes are slowed under pressure

Where is high pressure pertinent to microbes? Deep sea!

-abyssal environments are thought of as barren deserts, puncutated by reducing environments such as hydrothermal vents, cold deeps, and whale falls

-barotolerant deep-sea organisms tolerate $\geq$ 1300 atm; deepest ocean environment known is Challenger Deep of the Mariana Trench (1,100 atm)

-barophile (piezophile): Membranes and enzymes depend on high pressure to maintain their three-dimensional, functional shape. Sudden decompression by rupture cells (release of gas bubbles)

Where else is high pressure pertinent to microbes? Deep subsurface: pore water in rocks!

Osmotic Pressure affects microbial growth

- **Osmotic pressure** is the pressure exerted on a semipermeable membrane by a solution containing solutes that cannot freely cross membrane; related to concentration of dissolved molecules and ions in a solution

- All microbes cope with osmotic pressure

- Life evolved in a marine environment; hypotonic is "new"

- **Hypotonic** solutions (e.g. fresh water) have lower solute concentrations; cells placed in these solutions will **swell and burst**

  - Cell wall prevents bursting
  - Pressure buildup from inside cell prevents influx of additional water
  - Water can be actively pumped out; e.g. contractile vacuoles of some protozoans pump out excess water

Osmotic Pressure

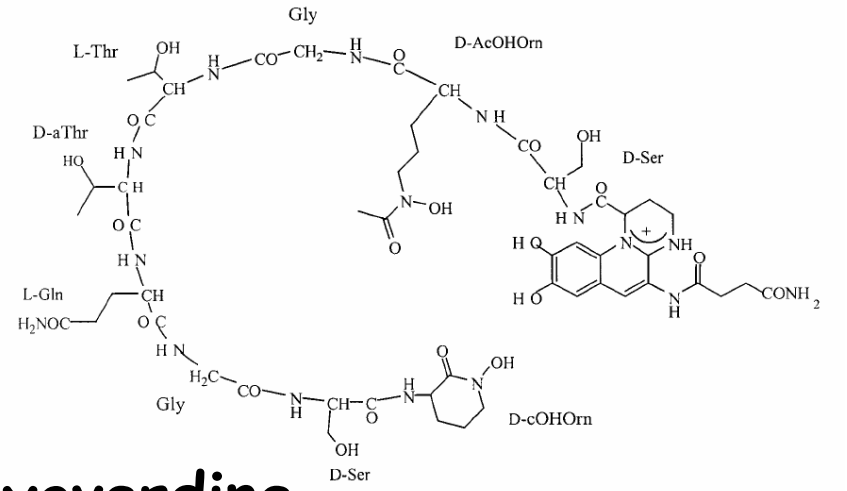
- **Hypertonic** solutions have greater solute concentrations; cells placed in these solutions will undergo **plasmolysis** (shriveling of cytoplasm)
  - Osmotic pressure dehydrates microbial cells
  - This effect helps preserve some foods comprising highly concentrated solutions: honey, molasses, corn syrup
  - Osmotolerant/osmophilic organisms avoid dehydration by balancing osmotic pressure with compatible solutes
  - *Aspergillus* and *Penicillium* (fungi) are osmotolerant
- **High salt solutions**, in addition to dehydrating cells, also **denature proteins**.
  - Salt lakes in arid environments where evaporation exceeds freshwater flow
  - Landlocked lagoons, tidal evaporation flats
  - Biota in high salt environments limited to halophilic and halotolerant species
  - Obligate halophiles – grow in up to 30% salt
  - Facultative halophiles – can tolerate high salt concentrations

Microbes adapt to nutrient stress, too…

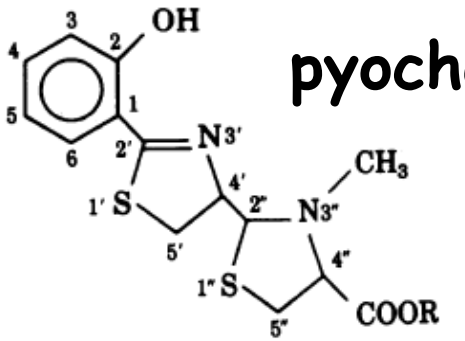
Pseudomonas aeruginosa synthesizes two siderophores, pyochelin and pyoverdine.

Pyochelin binds other transition metals, such as Cu(II), Co(II), Mo(VI), and Ni(II), with appreciable affinity. Repressed by high concentrations of these metals.

Pyoverdine binds Fe(III) with great affinity. Induced by low iron conditions.



pyoverdine *Fig. 1.* Structure of the pyoverdin **II**.



pyochelin

1: R = H

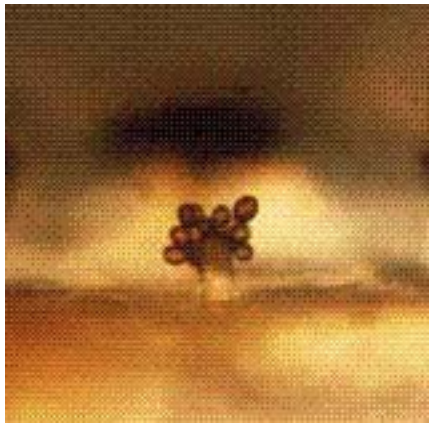
2: R = CH_3

Visca et al., 1992. Appl. Environ. Microbiol. .58: 2886

Cooperation among cells in a population: **myxobacteria**.

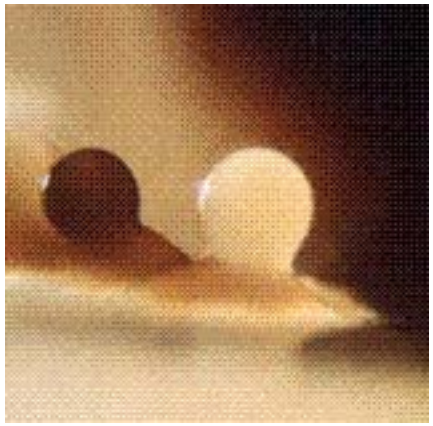
The myxobacteria are Gram-negative, ubiquitous, soil-dwelling bacteria that are capable of multicellular behavior. In the presence of nutrients, "swarms" of myxobacteria feed cooperatively by sharing extracellular digestive enzymes, and can prey on other bacteria. **When the food supply runs low**, they initiate a complex developmental program that culminates in the production of a fruiting body composed of hundreds of thousands of cells. The myxobacteria communicate with each other, and coordinate their movements through a cell-contact-dependent signal.

--Adapted from Dale Kaiser, 2003. Nat. Rev. Microbiol. 1:45



Stigmatella aurantiaca

Myxococcus stipitatus



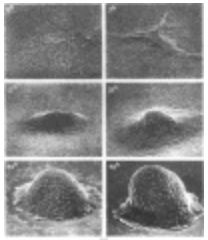
Stigmatella aurantiaca

PHOTOS COURTESY OF HANS REICHENBACH

Myxococcus xanthus coordinates cell movement with C-factor

C-factor helps organize the movement* of cells:

- rippling
- aggregation
- end-to-end packing in "rafts" inside spores.



JM Kunder and D. Kaiser, 1982

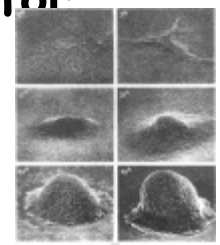
C-factor is a small (20 kD), membrane-bound protein
C-factor is NOT diffusible.
C-factor requires cell-cell contact
C-factor autoinduces, and there are thresholds of C-factor for each subsequent developmental stage

*Gliding motility in *M. xanthus* involves two different "gliding machines", one at each cell pole:

1. the S-machine, which depends on type IV pili
2. the A-machine, which seems to involve a slime extrusion mechanism.

C-signal induces cells to move with increased gliding speeds, in longer gliding intervals and with decreased stop and reversal frequencies… increasing travel rates of cells.

Myxococcus xanthus signals starvation with diffusible A-factor resulting in aggregation



JM Kunder and D. Kaiser, 1982

Cells sense that nutrient density is getting low, and release **A-factor**

A-factor
-mix of 6 amino acids (result of proteolysis): trp, pro, phe, tyr,leu, ile
-amino acids are 10-fold less than concentration necessary to support growth
-only released by starving cells
-each cell only releases a fixed amount of A-factor
-A-factor is diffusible

If enough cells release A-factor, the population "agrees" to commit to fruiting body development, and begins the first stages of aggregation.

There's a time limit for fruiting body formation. During starvation, cells "sense impending doom" and seem to act proactively to fruit and disperse. (Why?).

Cells sense actual starvation as the lack of one or more amino acylated tRNAs, which triggers the "**stringent response**" during which protein synthesis is temporarily shut down, so no fruiting body could be formed.

The Stringent Response

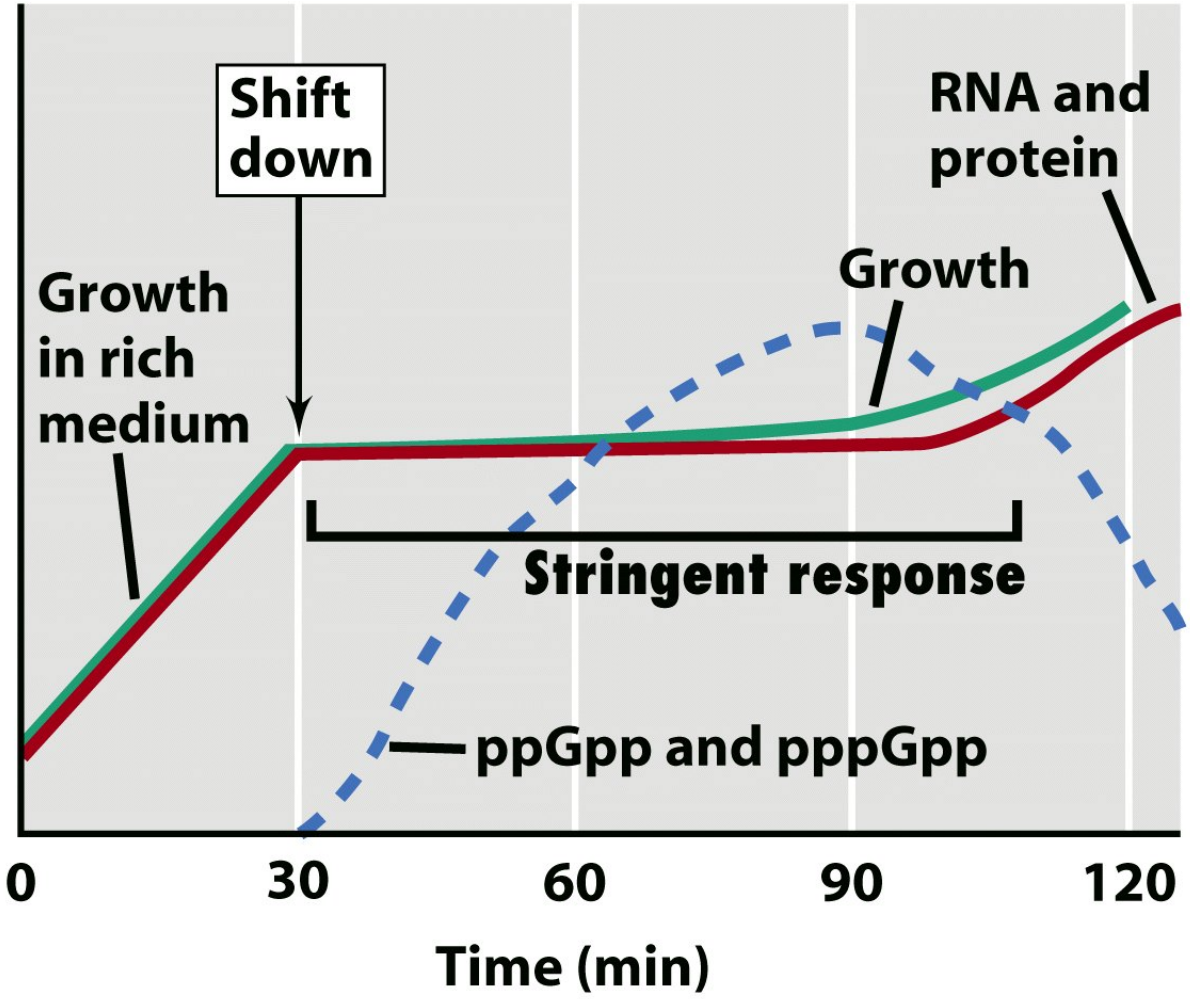


Figure 8-21a Brock Biology of Microorganisms 11/e

Repressed: rRNA and tRNA synthesis and genes for ribosomal proteins

Induced: amino acid biosynthetic operons and certain pathways for macromolecular precursors that are sparse; protease dismantles unassembled ribosomal proteins

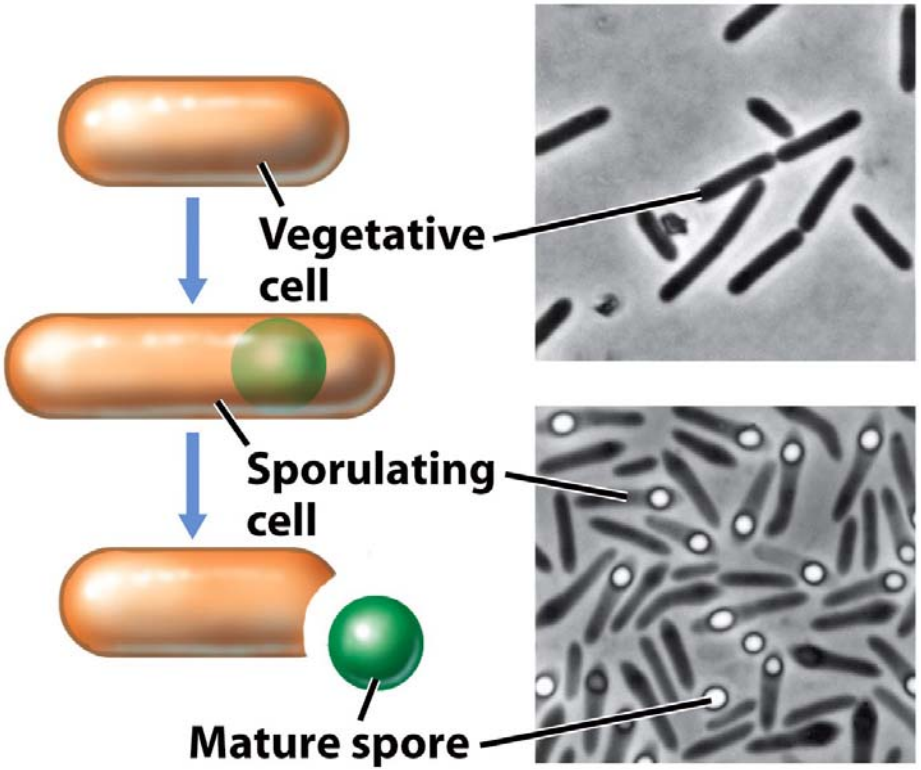
Outcome: re-setting of protein production to fit available nutrients in environment.

Bacillus and endospore formation

First, "food wars" – killing by some cells of others

-intense competition/cannibalism or "multicellular" apoptosis during development? (Sporulation may occur in wild at apical tips as in *Myxococcus)*

Next, irreversible program to sporulate by some cells



What happens to non-spore formers stress occurs?

VBNCs

VBNCs (controversial – not yet cultured is better), quiescent, somnicells, resting cells, cryptically growing cells

Changes in cellular morphology, concentration/structure of major polymers (proteins, membrane lipids, nucleic acids), inability to grow on medium that supported growth prior to shock

Dormant state: Metabolically active (stained by acridine orange) but not culturable on any plating medium or broth.

Ultramicrobacteria

Following C or multiple nutrient starvation, small, coccoid cells are observed (increased S:V ratio)

Pass through 0.45 μM and 0.2 μM filters

ultramicrobacteria, dwarf cells, ultramicrobacteria, volumetrically challenged microorganisms.

-Reductive division (no increase in cell size before binary fission)

-Gradual "shrinking" without replication

Cells ≤ 0.3 μM common (> 70%) in soil, seawater, subsurface. Missed by culturing and simple microscopy. Seen via fluorescence microscopy, SEM.

Note: following N, P starvation, cells swell rather than shrinking

Lower limits of cell size

Nanobes/nanobacteria and nanofossils: 0.01 to 0.2 μM "cells" found in geologic materials.

Folk, R.L. 1993. SEM imaging of bacteria and nanobacteria in carbonate sediments and rocks. J. Sed. Petrol. 63: 990-999.

-posited that nanobes are responsible for much of mineral precipitation; **may** comprise majority of Earth's biomass

****

McKay et al. 1996. Search for past life on Mars: possible relic biogenic activity in Marian meteorite ALH 84001. Science 273: 924-930.

-Observed small ovoid features, 20 to 100 nM (0.02-0.1 μM) in diameter, visible in SEM.

Do nanobes really exist?

Arguments for and against …

For: incredibly high S:V ratio for nutrient exchange in limiting environment

Against: Cell must be large enough to house macromolecules. Individual prokaryotic ribosomes are 20-25 nM in diameter, active cells have 100's or more.

Psenner and Loferer: **0.3 μM** (300 nM) diameter required to house **enough macromolecules** for life.

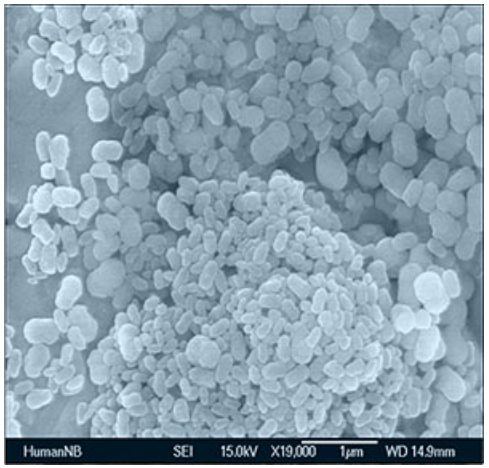
Maniloff: Same argument; **0.14 μM** (140 nM) diameter limit

K. Nealson: metabolically active cells need **sufficient concentration of metabolites in cytosol.** mM to mM concentrations of such metabolites require **0.1 μM (100 nM)** diameter.

For: Fewer macromolecules needed if cells more efficient (higher enzyme V_{max}, fewer gene redundancies)

Against: most microbes (except intracellular parasites) are not streamlined, need large genomes to encode "catastrophe kit" to handle adversity and nutrient deprivation. Extra genes, membrane sensory proteins, etc.

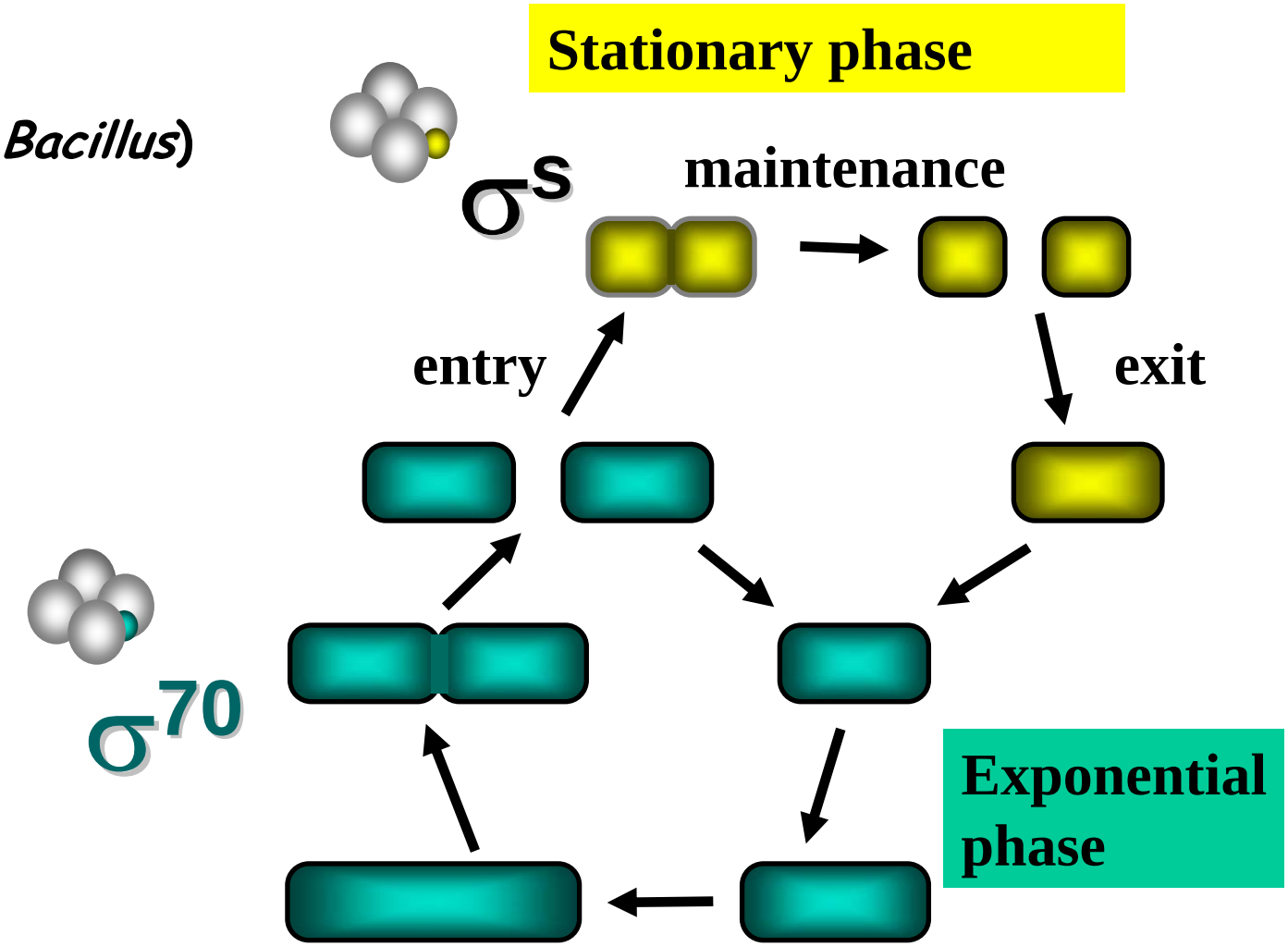
12/2006: Calcification due to nanobacteria (0.3 μM or 300 nM) diameter) in kidneys? Fits even most stringent hypothetical size limits



Bacterial life cycle: sigma factors drive different subsets of genes

σ^S (*E. coli*) or σ^B (*Bacillus*) activated by:

Energy depletion
Ethanol
Salt
Acid
Alkalinity
Heat
Oxidative stress



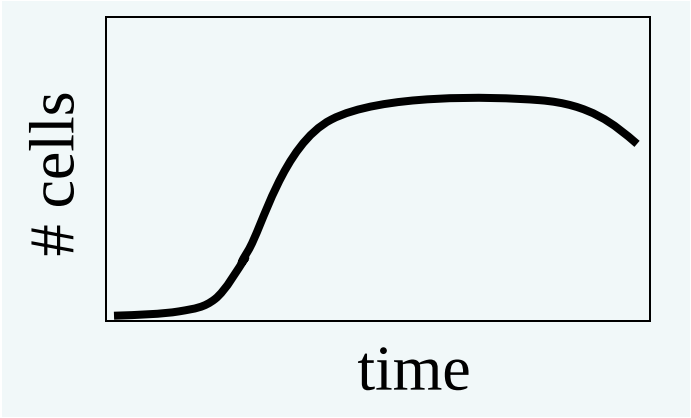
Stationary phase in *Bacillus* - low nutrient levels:

- cease growth, de-repress amino acid biosynthetic genes (**stringent response**)

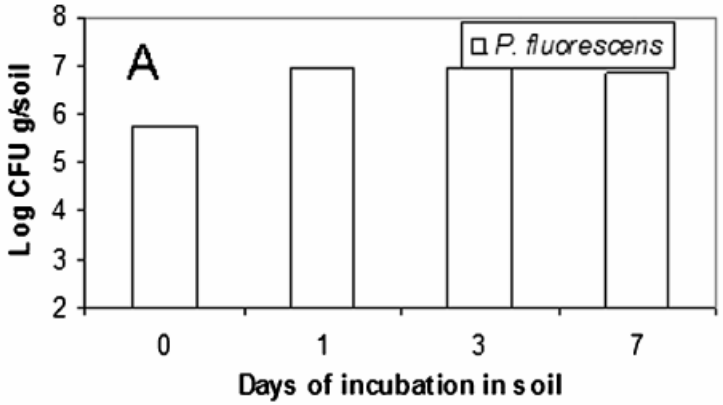
-activate uptake and catabolism of different C sources (**catabolite repression**)

-activate genes for DNA repair
-activate cell differentiation: spores!

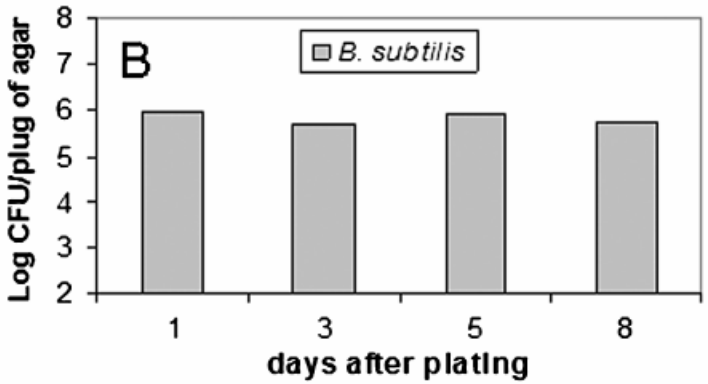
➔400 proteins cease to be expressed
➔150 new proteins begin to be synthesized



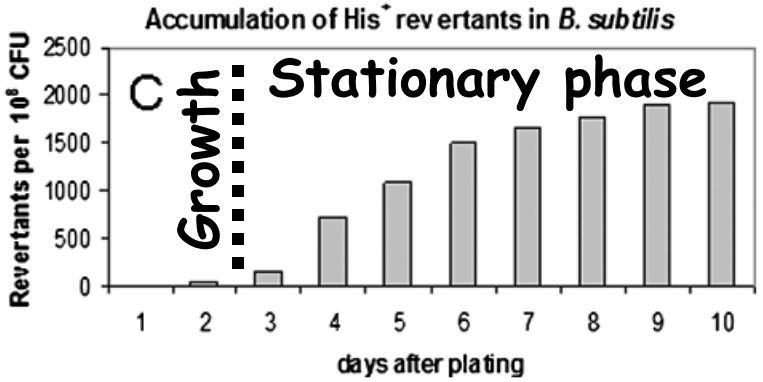
Stationary phase mutagenesis in soil: adaptive mutation?



Soil is a complex habitat that subjects bacterial cells to conditions starvation/nongrowing conditions.



Nutritional stress mimicked by laboratory media lacking a required nutrient: no growth (stationary phase)



Bacterial populations carrying amino acid auxotrophies held in stationary phase accumulate prototrophic revertant colonies over time.

Robleto et al., 2007. Crit. Rev. Biochem. Molec. Biol. 42: 327

σ^s enhances mutation?

In *E. coli*, σ^S-deficient strains show a decrease in accumulation of "adaptive mutants" via hypermutation.

-Cells under arrested growth experience higher mutation because:

high level of oxidative damage to DNA

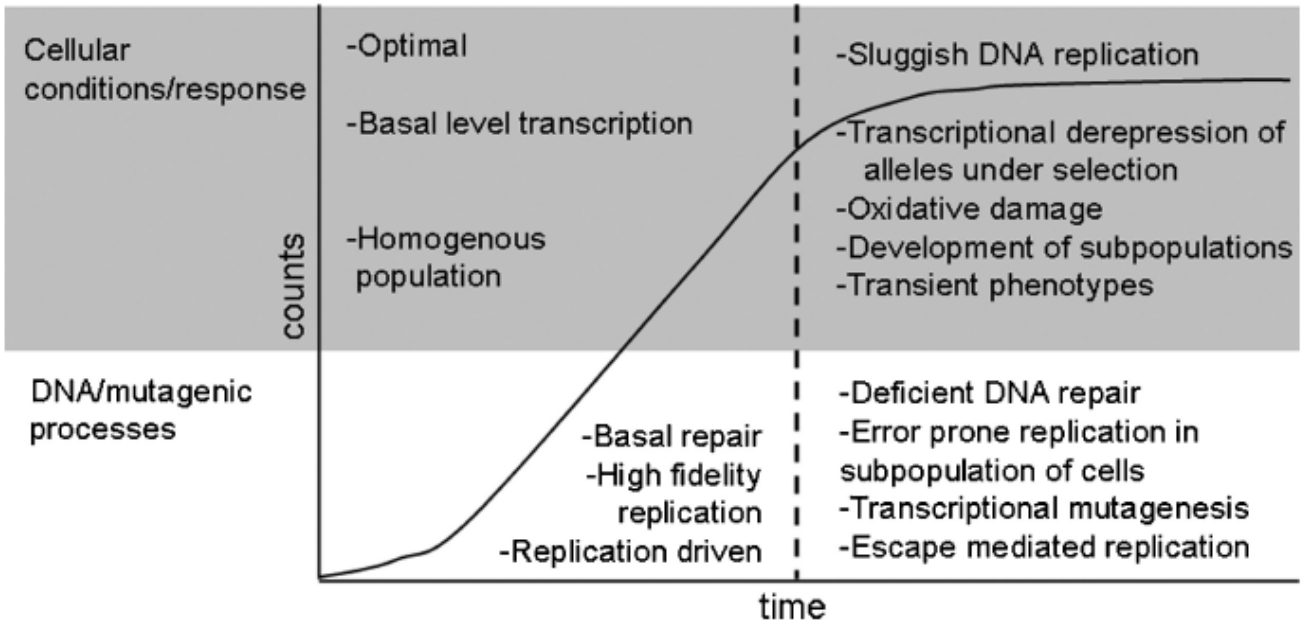
DNA repair systems are limiting/repressed (e.g. *mutSL* operon)

Uptake of foreign DNA (*Bacillus* and other naturally competent species; ~ 10% of total population does this)

-Pol IV is thought to be involved in DNA repair of ds breaks and nicks but is more error-prone than housekeeping DNA Pol I.

-σ^S controls switch to error-prone polymerases, including Pol IV, confining mutations to a non-growth (not DNA replication) time in the life cycle.

Stationary phase in *B. subtilis:* conditions, cellular responses, and mutagenic processes



Robleto et al., 2007. Crit. Rev. Biochem. Molec. Biol. 42: 327

Transcriptional mutagenesis

1. Mutation (e.g. point mutation) or lesion (e.g. thymine dimer) forms in DNA

2. RNA polymerase is unimpeded by mutation; easily slips over lesion (Mfd and Gre proteins rescue stalled transcription elongation complexes)

3. Mutant protein results

4. If protein is advantageous, e.g. permits catabolism of a carbon source that could not previously be used, growth ensues.

5. Stationary phase is lifted, replication begins

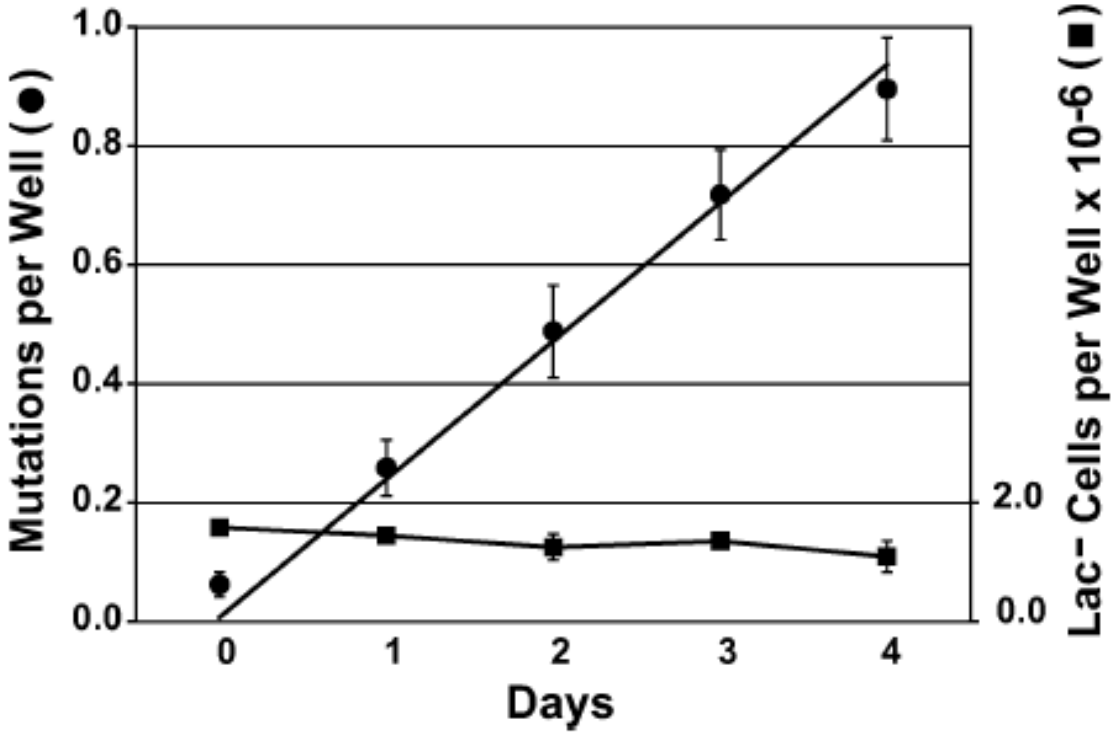
6. If repair does not occur on DNA before the replication fork arrives at the mutated site, the mutation is fixed in daughter cell's DNA.

Term	Definition
Directed mutation*	The appearance among cells under selection of mutations that relieve the selective pressure in the absence of the appearance of nonselected mutations.
Adaptive mutation	The appearance among cells under selection of mutations that relieve the selective pressure whether or not other nonselected mutations are also produced.
Stress-induced mutation	The induction of a general mutagenic state in response to stress. If the stress is starvation, then the equivalent term is "starvation-induced mutation".
Transient mutation	A temporary state during which the mutation rate is increased, resulting in the accumulation of mutations throughout the genome. Could be the result of various inducible processes.
Hypermutation	A state during which the mutation rate is increased to extremely high, potentially lethal, levels resulting in the accumulation of mutations throughout the genome.
Stationary-phase mutation	A general mutagenic state that occurs in cells in stationary phase.
Stationary phase	The period of laboratory batch culture when the cells have run out of one or more nutrients. Also used to describe nutrient-limitation in natural populations.
Long-term stationary phase	The period of laboratory batch culture after the cells have entered stationary phase and after the majority (*e.g.*, 99% or more) have died. Characterized by balanced growth and death of the population.
Starvation	A general term describing the lack of one or more nutrients required for growth. Starvation can result from the normal depletion of nutrients during batch culture, or from a population being artificially suspended in nutrient-free medium.
Nutrient-limitation; nutritional-deprivation	Same as starvation, but often implies that only one nutrient is lacking.
Hunger	The state of cells when they are growing with suboptimal levels of nutrients, as for example in nutrient-limited chemostats.
Nutritional selection	A nutrient limitation that can be overcome by mutation; *e.g.*, Lac^- cells incubated with lactose as the only carbon and energy source.
Adaptive evolution	Evolution that proceeds by selection for improved fitness, in contrast to evolutionary changes caused by neutral mutations and genetic drift.

Examples of stress-induced mutation

Name†	Organisms	Type of Mutation	Selected Phenotype	Genetic Requirements	References
Starvation-induced Mu-mediated fusions	*E. coli*, Mu	Transposition	Growth on arabinose plus lactose	RpoS, ClpP, HNS*	(Cairns *et al.*, 1988; Foster *et al.*, 1994a; Gomez-Gomez *et al.*, 1997; Lamrani *et al.*, 1999; Maenhaut-Michel *et al.*, 1994; Maenhaut-Michel *et al.*, 1997; Mittler *et al.*, 1990; Shapiro, 1984)
Resting organisms in a structured environment (ROSE) mutagenesis	*E. coli*	Base substitutions	Resistance to rifampicin	CyaA. RecA. LexA*, UvrB, Pol I, Not Pol V, Not RecBCD, Not RpoS	(Taddei *et al.*, 1995; Taddei *et al.*, 1997a)
Mutagenesis in aging colonies (MAC)	*E. coli*	Base substitutions	Resistance to rifampicin	RpoS, Crp, CyaA, RecA, MMR*, Pol II, Not RecBCD, Not Pol I	(Bjedov *et al.*, 2003)
SOS-dependent spontaneous mutagenesis	*E. coli*	Base substitutions	Tryptophan prototrophy	RecA, Pol V	(Bhamre *et al.*, 2001; Timms *et al.*, 1999)
Stationary-phase mutagenesis	*P. putida*	Frameshifts, base substitutions, transposition	Growth on phenol	Pol IV, Pol V, RpoS, MutY, Not RecA, Not MMR*	(Ilves *et al.*, 2001; Kasak *et al.*, 1997; Saumaa *et al.*, 2002; Saumaa *et al.*, 2006; Tark *et al.*, 2005; Tegova *et al.*, 2004)
Stationary-phase mutagenesis	*B. subtilis*	Base substitutions	Amino acid prototrophy	ComA, ComK, Pol IV, MMR*, Mfd, Not RecA, Not RpoS analog	(Pedraza-Reyes *et al.*, 2004; Ross *et al.*, 2006; Sung *et al.*, 2002; Sung *et al.*, 2003)
Adaptive mutation	*E. coli*	Frameshifts	Growth on lactose	Pol IV, RecA, RecBCD, RpoS, GroE, Ppk, Not Pol V	See text

Accumulation of Lac+ mutations in liquid minimum lactose medium.

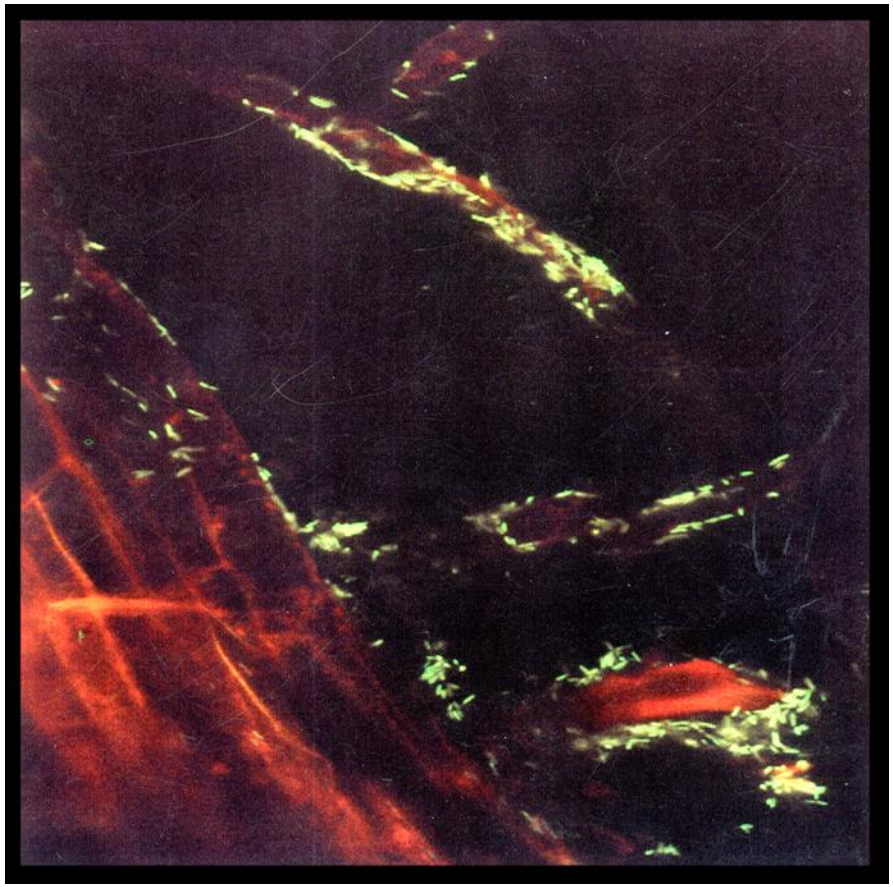


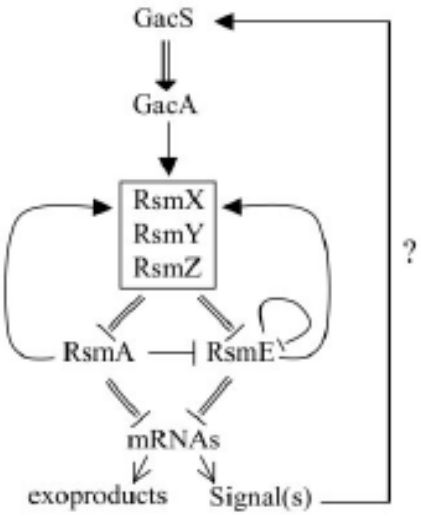
~10^6 cells/100 μl minimum lactose medium previously "scavenged" of any non-lactose carbon sources by incubating for 3h with 10^9 Δ(*lac*) cells per mL. Those cells were removed by centrifugation followed by filtration.

Foster, P. 1994. Genetics 138:256.

Other methods for "adaptive" mutation:

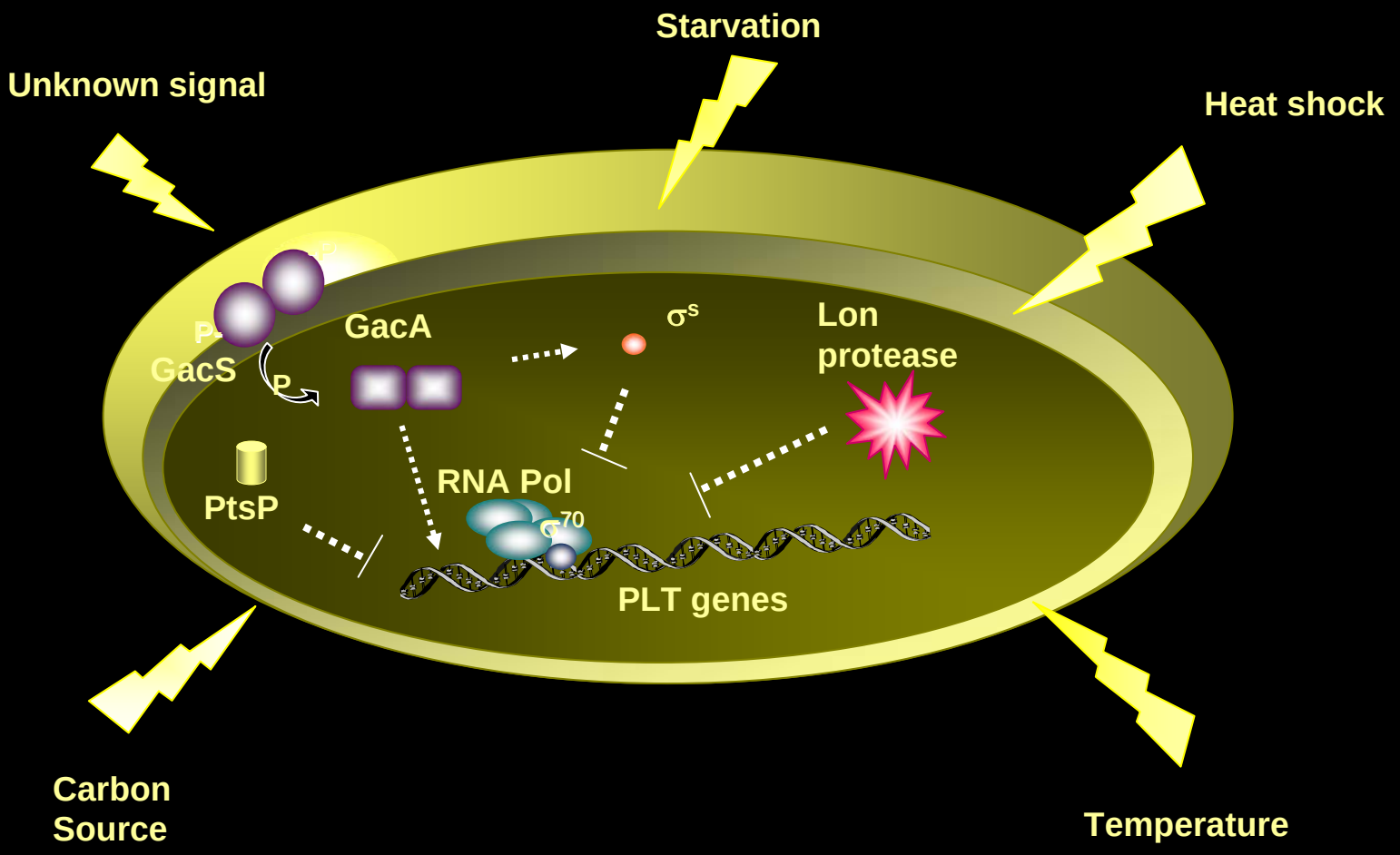
Pseudomonas and other rhizosphere bacteria secrete enzymes and toxins and "deal" with environment when GacAS is activated



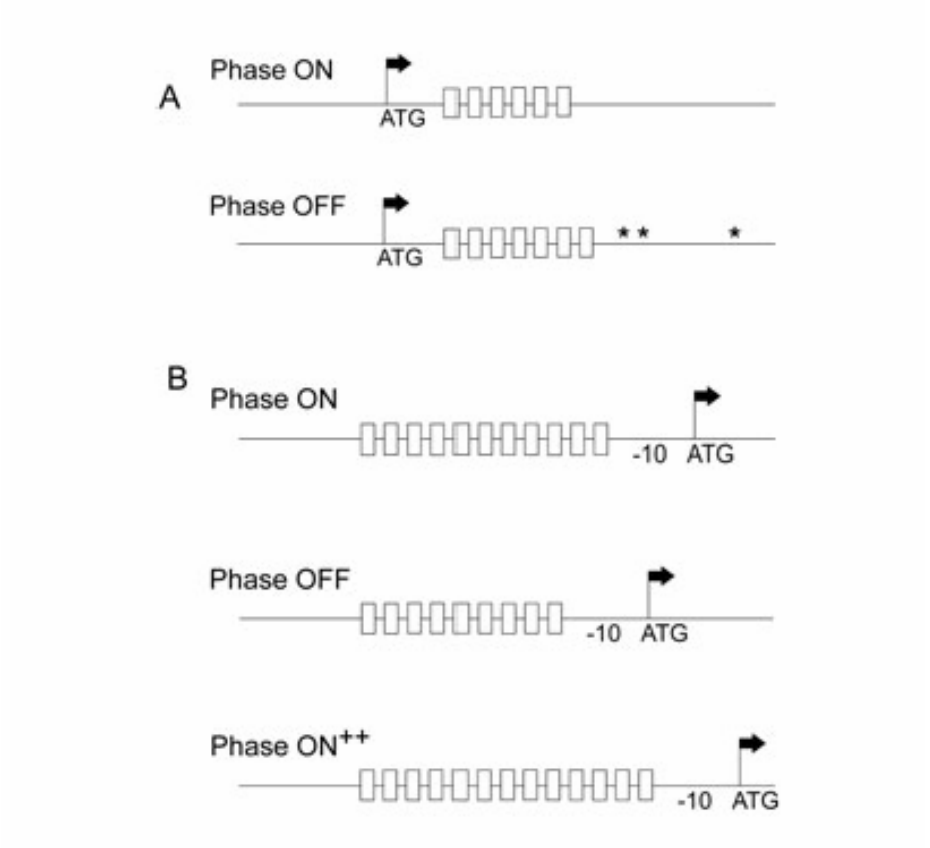


Kay et al., 2005. PNAS 102: 17236.

Factors known to influence exoproduct biosynthetic gene transcription in *Pseudomonas*

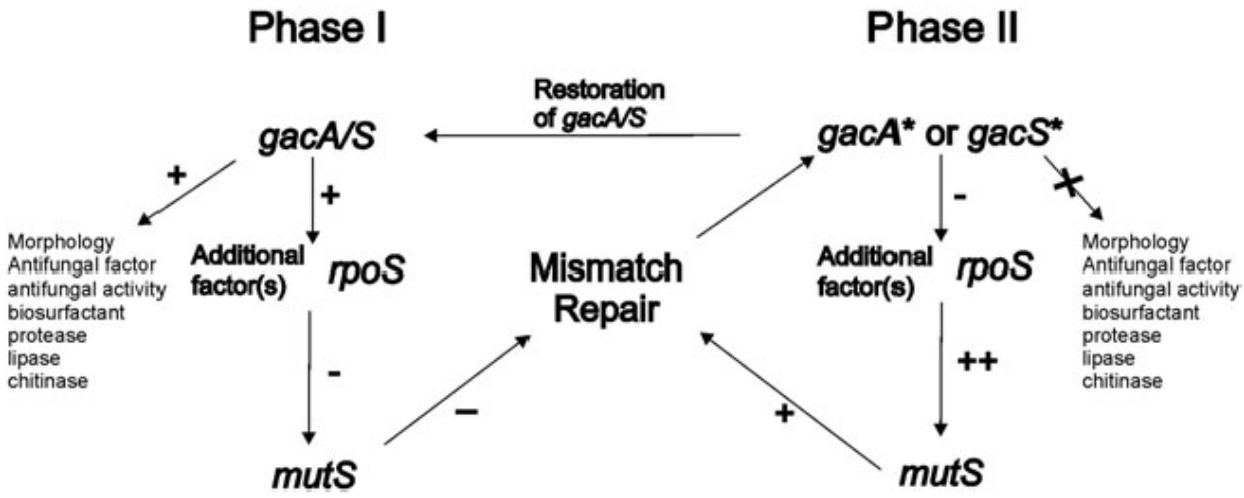


Model for phase variation via slipped-strand mispairing



van den Broek, D., 2005. Environ. Microbiol. 7:1686

Model for the genetic regulation of spontaneous mutations accumulating in *gac*



van den Broek, D., 2005. Environ. Microbiol. 7:1686