Lecture Supplements

Plant Bacteriology

Microbial (Bacterial) Ecology

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Table of Contents

- Definitions
- Course synopsis
- Proceedings/Reviews/Monographs/Book chapters/PowerPoints/PDF files
- Books on microbial ecology
- Journals
- Types of Microorganisms
- Cell size
- Key terms
- Research topics and expertise
- Metagenomics
- Human microbiome
- Human holobiome
- Microbiome and metabolic activity
- Prebiotic vs. probiotic
- Phytobiomes
- Quorum sensing
- Biofilm
- Ice nucleation bacteria
- Sea ice microbial ecology
- Selected References

Microbial ecology

Course synopsis

- Microbial Community
- Species
- Population
- Community
- Ecosystem
- Species diversity
- Autochthonous and Allochthonous microorganisms

- Dispersal of Microorganisms
  - Active transmission
  - Passive transmission
- Dispersal of microorganisms by:
  - Air
  - Soil
  - Water
  - Inanimate objects
  - Biological vectors

Ecosystem: Habitat plus community.
Soil Bacteria can be divided into two broad categories:
Autochthonous (indigenous) - true residents of the soil, which may have resistant stages (endospores, spores, cysts), and
Allochthonous (transients) - transients or invaders in the soil habitat, which enter the soil, diseased tissues, etc.

Microbial ecology
Course synopsis

- Colonization
  - Factors that aid colonisation of an environment by microorganisms
- Succession
  - Pioneer organisms
  - Climax
- Forms of succession:
  - Autogenic, and
  - Allogenic
- Factors affecting microbial succession in an environment.

- Microbial interactions:
  - Interaction within a population
  - Interaction within diverse population
  - Positive interactions
  - Negative interaction

Microbial ecology
Course synopsis

- Positive interactions:

1. Commensalism
2. Synergism
3. Mutualism
4. Neutralism

- Negative Interactions:

1. Amensalism
2. Parasitism
3. Predation

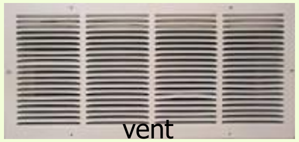
Dr Balogun, S.A. PhD
Environmental/Petroleum Microbiologist

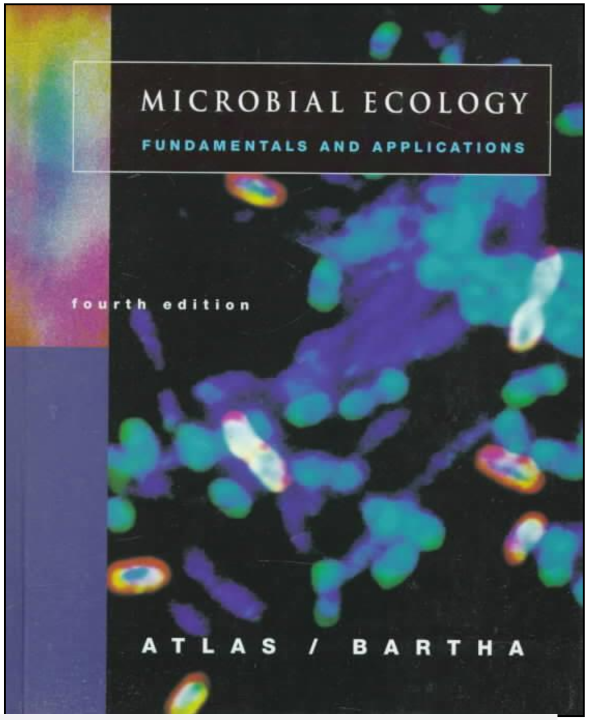
PowerPoints, Monograph, PDF files, Lecture Notes

- ENVH 4387/5387 Biological Analysis.ppt. Principles of Ecology and Microbial Ecology. Lecture Topic Number 1. 18 slides.
- Erinle, I. D.1987. Ecology and Survival of Plant Pathogenic Bacteria in the Semi-Arid Zones of Nigeria. Chapter. Plant Pathogenic Bacteria Volume 4 of the series Current Plant Science and Biotechnology in Agriculture. pp 763-763.
- Hirano, S.S. and C. D. Upper.1983. Ecology and Epidemiology of Foliar Bacterial Plant Pathogens. Annual Review of Phytopathology 21: 243-270.
- Klymnyuk, S.I. Microbial ecology. 71 slides.
- Polz, M. Evolution and Ecology of Pathogens. Civil & Environmental Engineering Massachusetts Institute of Technology. 40 slides.
- Rudney, J. DENT 5301. ppt. Introduction to Oral Biology. Oral Microbial Ecology. 20 slides.
- van Ooij, C. 2011. Bacterial ecology: Microbial communities hold the fort. Nature Reviews Microbiology 9, 312-313.

Microbial Ecology: Fundamentals and Applications

- Ronald Atlas and Richard Bartha
- Publisher: Benjamin Cummings; 4 edition (December 21, 1997)
- 640 pages.

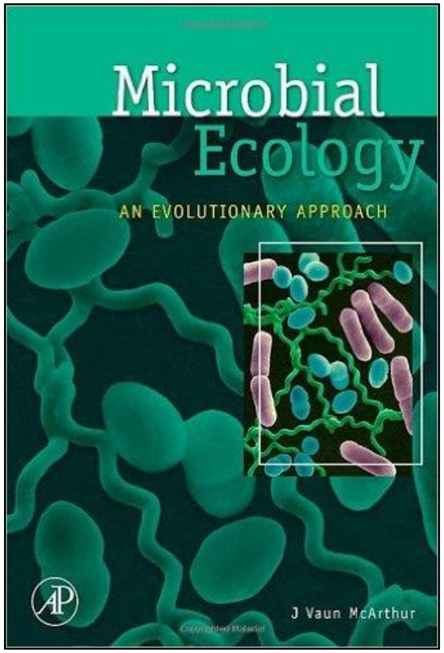




The authors incorporate the latest research and literature sources as well as new and expanded coverage of hot topics such as biofilms, thermal vent communities, extreme habitats, starvation response, molecular methods for studying microbial ecology, microbial diversity, biodegradation, and bioremediation.

Microbial Ecology: An Evolutionary Approach

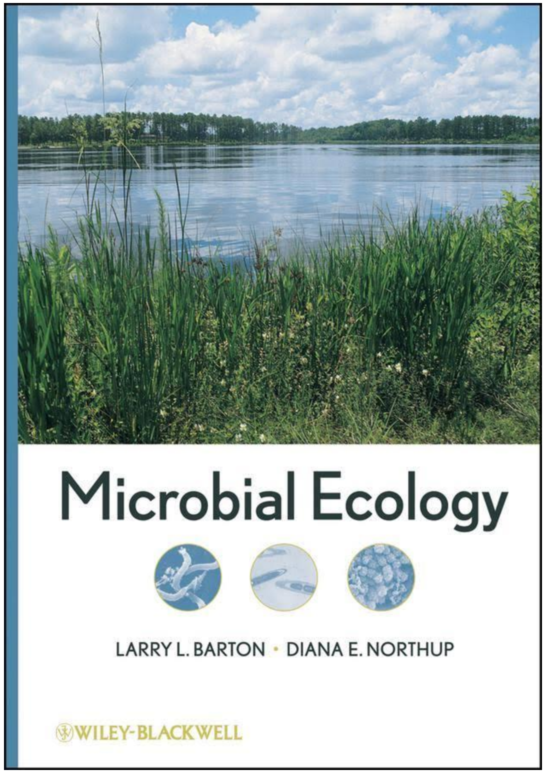
- J Vaun McArthur
- Publisher: Academic Press; 1 edition (February 15, 2006)
- 432 pages.



Microbial Ecology examines microbiology through the lens of evolutionary ecology. Measured from a microbial perspective, this text covers such topics as optimal foraging, genome, reduction, novel evolutionary mechanisms, bacterial speciation, and r and K selection. Numerous aspects of microbial existence are also discussed and include: species competition, predation, parasitism, mutualism, microbial communication.
The two evolutionary "strategies" are termed r-selection, for those species that produce many "cheap" offspring and live in unstable environments and K-selection for those species that produce few "expensive" offspring and live in stable environments.

Microbial Ecology

- Larry L. Barton and Diana E. Northup
- Publisher: Wiley-Blackwell; 1 edition;
- October 4, 2011;
- 440 pages.

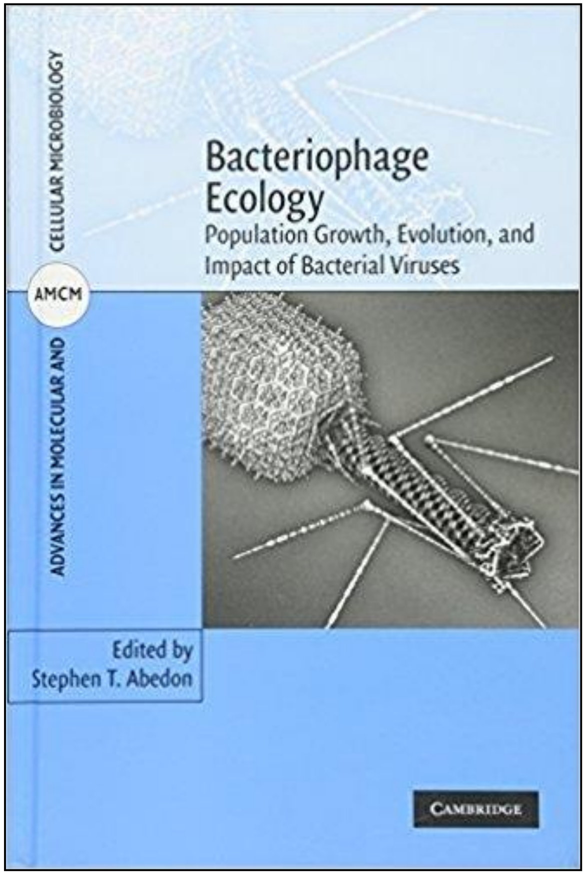


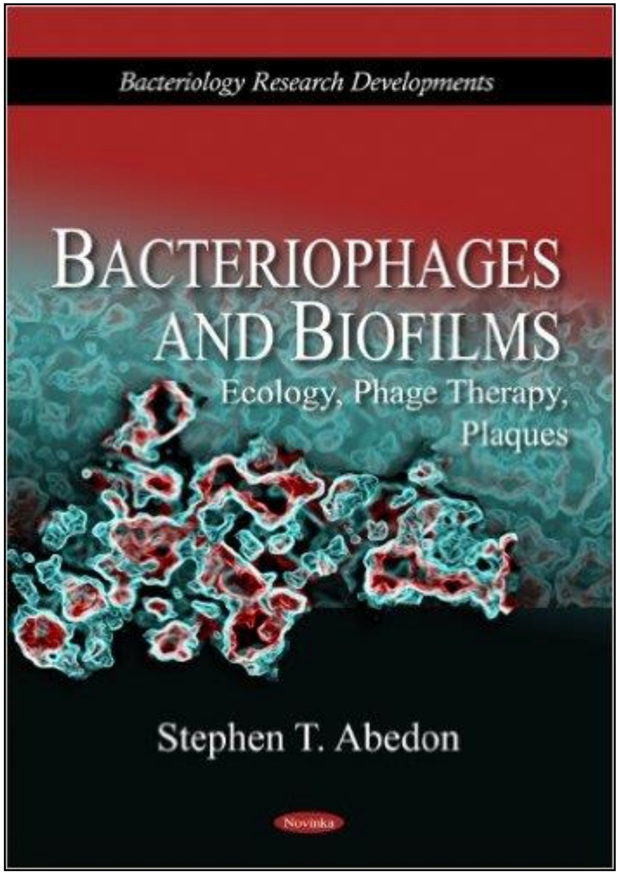
This book covers the ecological activities of microbes in the biosphere with an emphasis on microbial interactions within their environments and communities.

Books

Microbial Ecology

1. Bacteriophage ecology,2008
2. Bacteriophages and Biofilms: Ecology, Phage Therapy, Plaques,2011

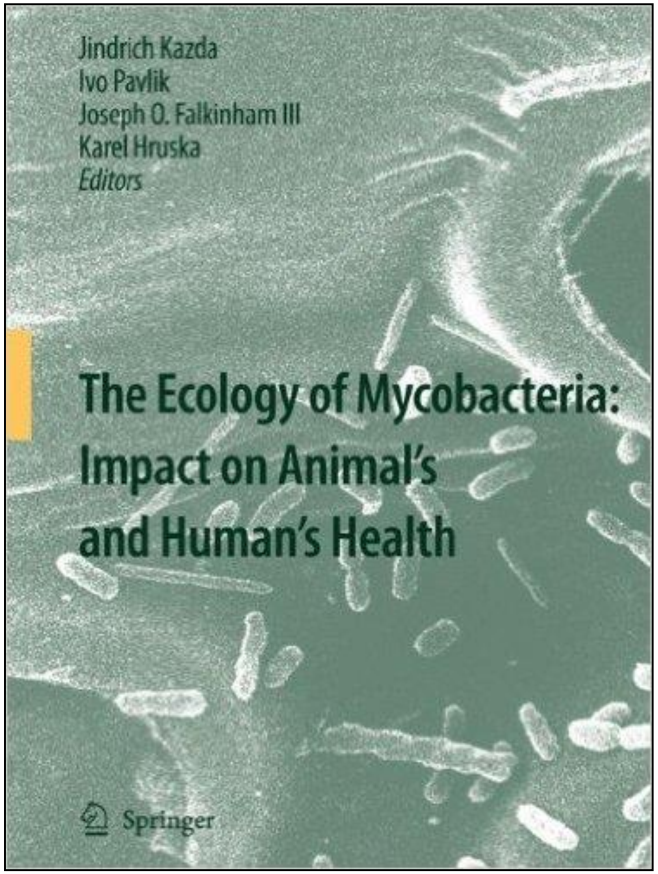


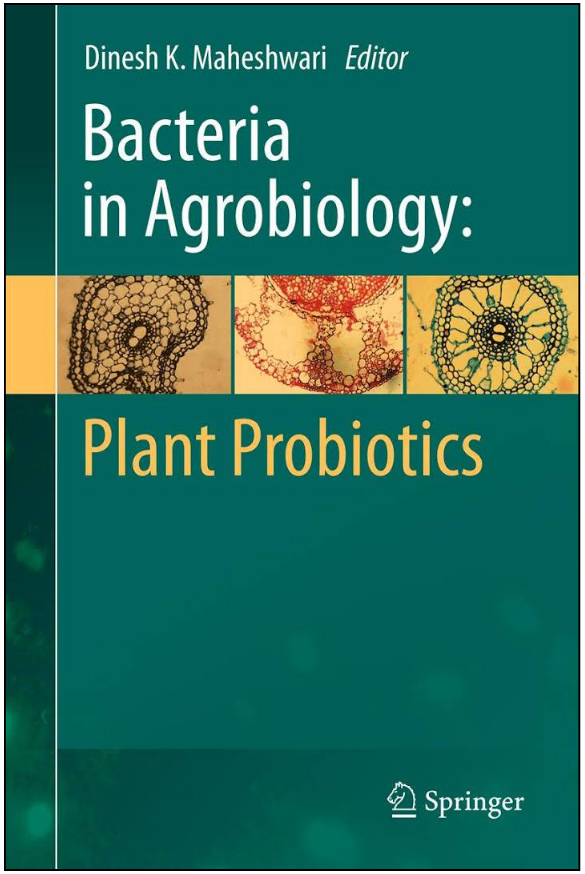


Books

Microbial Ecology

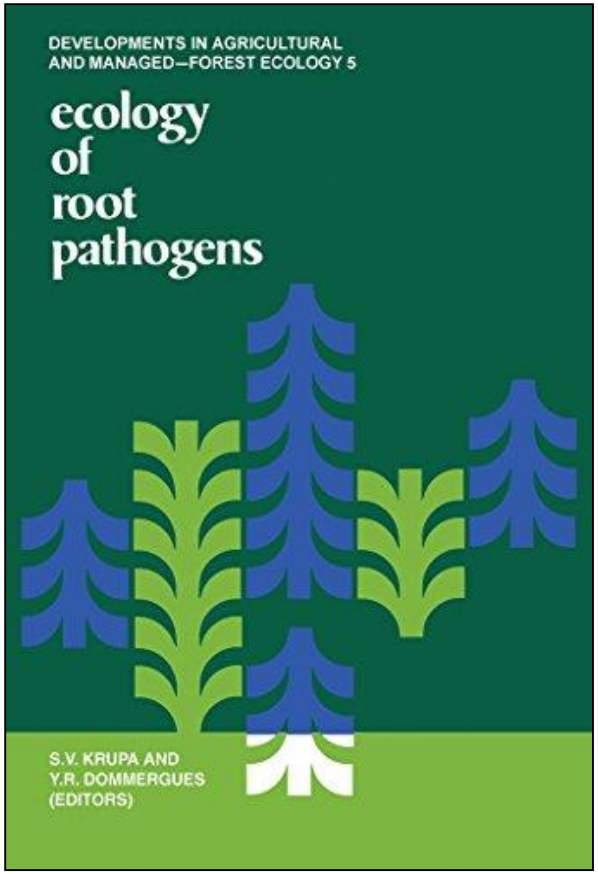
1. Bacteria in agrobiology: Plant probiotics,2012
2. The Ecology of Mycobacteria: Impact on Animal's and Human's Health,2009





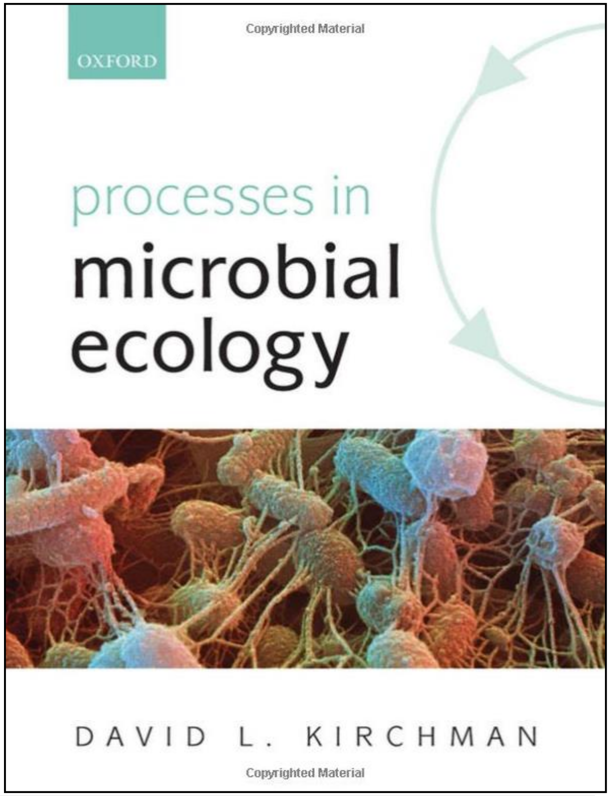
Ecology of Root Pathogens

- **Ecology of Root Pathogens (Developments in agricultural and managed-forest ecology).**
- Editor: S. V. Krupa and V.R. Dommergues.
- Publisher: Elsevier (December 2, 2012);
- 281 pages.



Processes in Microbial Ecology

- **Processes in Microbial Ecology**
- Dave Kirchman
- Publisher: Oxford University Press; 1 edition (March 24, 2012);
- 328 pages.

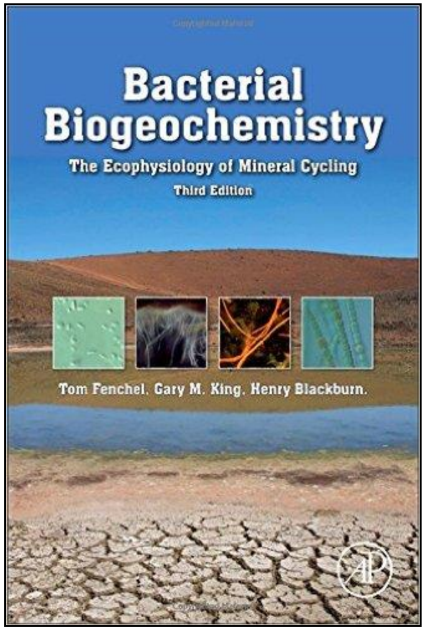


Processes in Microbial Ecology discusses the major processes carried out by viruses, bacteria, fungi, protozoa, and other protists in freshwater, marine, and terrestrial ecosystems.

Books

Bacterial Biogeochemistry:
The Ecophysiology of Mineral Cycling

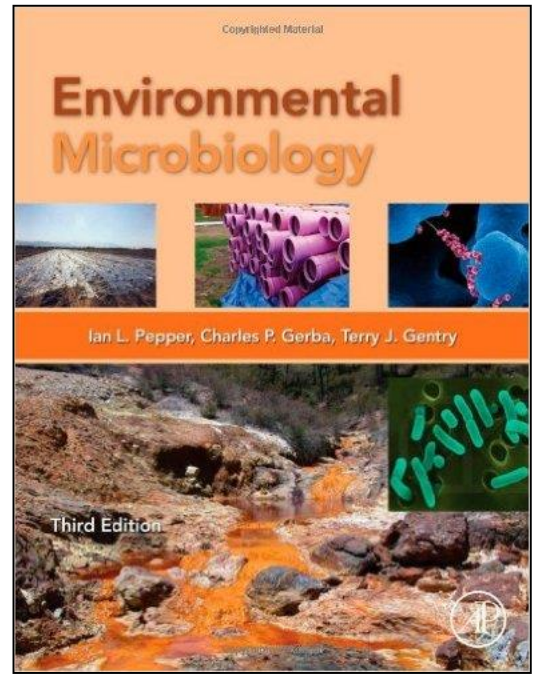
- Tom Fenchel, Gary M. King and Henry Blackburn
- Publisher: Academic Press; 3 edition (August 10, 2012);
- 312 pages.



Focuses on bacterial metabolism and its relevance to the environment, including the decomposition of soil, food chains, nitrogen fixation, assimilation and reduction of carbon nitrogen and sulfur, and microbial symbiosis. Also covers bioenergetic processes, characteristics of microbial communities, spatial heterogeneity, transport mechanisms, microbial biofilms, extreme environments and evolution of biogeochemical cycles.

Environmental Microbiology

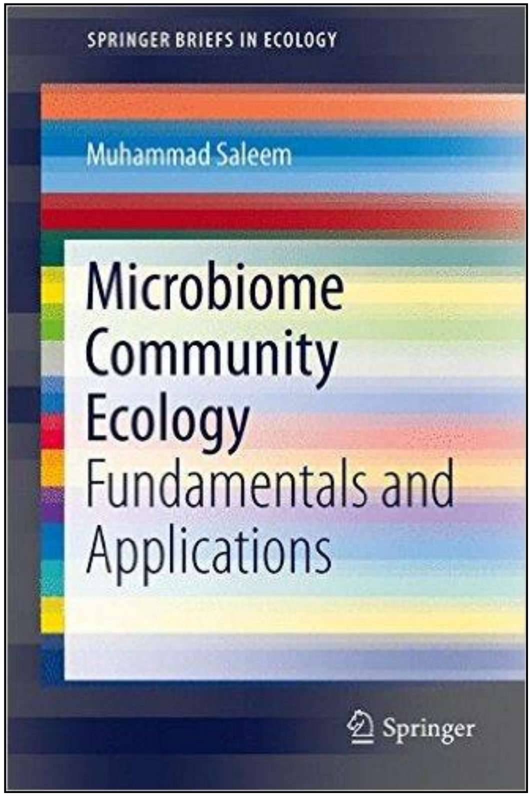
- Ian Pepper, Charles P. Gerba and Terry J. Gentry
- Publisher: Academic Press; 3 edition (April 7, 2014);
- 728 pages.



Covers from terrestrial and aquatic ecosystems to urban and indoor environments. Discussing biogeochemical cycling, bioremediation, environmental transmission of pathogens, microbial risk assessment, and drinking water treatment and reuse. The final chapter highlights several emerging issues including impact of climate change on microbial infectious disease, and the development of antibiotic-resistant bacteria.

Microbiome Community Ecology
Fundamentals and Applications

- Muhammad Saleem with contributions of M Brian Traw; Zahida H Pervaiz.
- Springer Briefs in Ecology
  Publisher: Springer; 2015 edition (January 28, 2015)
- 152 pages.

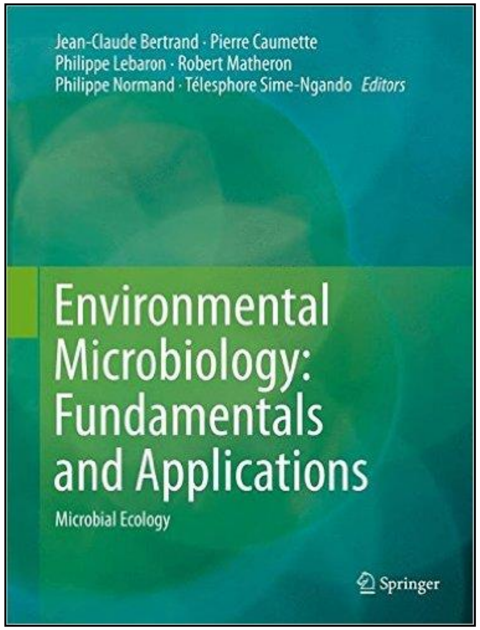


"Microbiome Community Ecology" aims to bring prokaryotes into the focus of ecological and evolutionary research, especially in the context of global change.

Books

Environmental Microbiology: Fundamentals and Applications

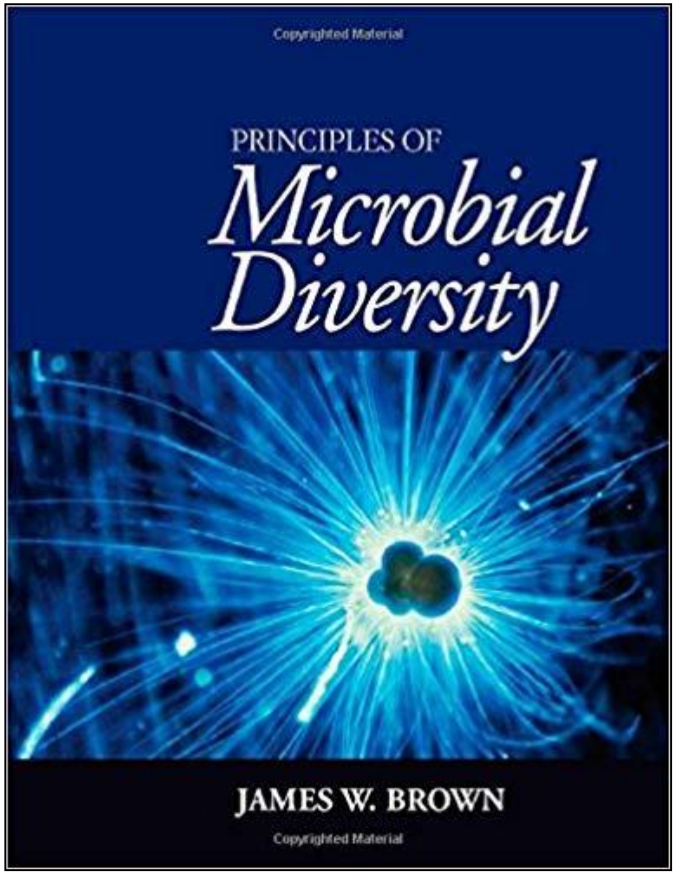
- Bertrand, J. C., Caumette, P., Lebaron, P., Matheron, R., Normand, P., Sime-Ngando, T. (Eds.)
- Publisher: Springer; 2015 edition (January 27, 2015);
- 933 pages.



This book emphasizes on study tools, microbial taxonomy and the fundamentals of microbial activities and interactions within their communities and environment as well as on the related food web dynamics and biogeochemical cycling. The work exceeds the traditional domain of microbial ecology by revisiting the evolution of cellular prokaryotes and eukaryotes and stressing the general principles of ecology.

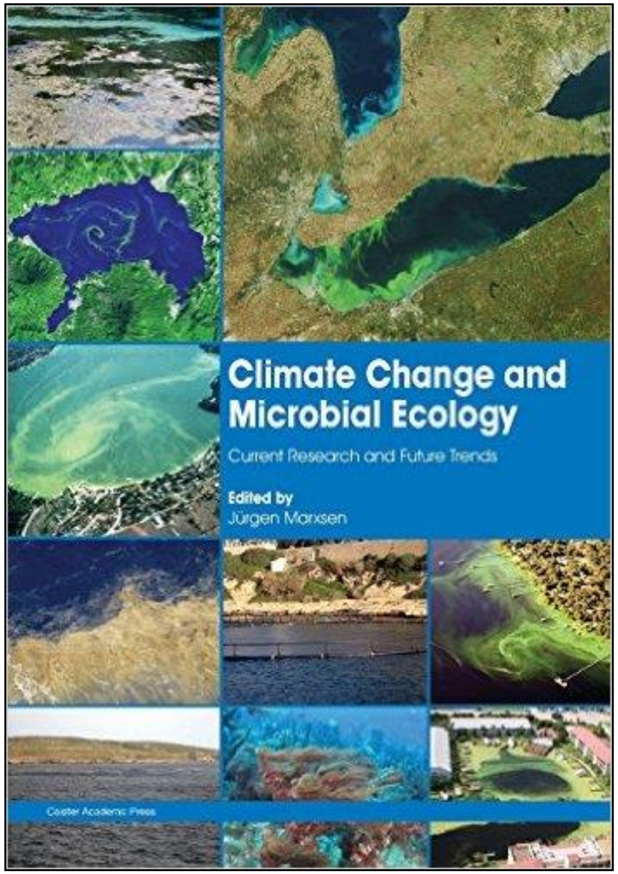
Principles of Microbial Diversity

- **Principles of Microbial Diversity**
- James W. Brown
- Publisher: Wiley;
- ASM Press; 1st edition (December 16, 2014)
- 406 pages.



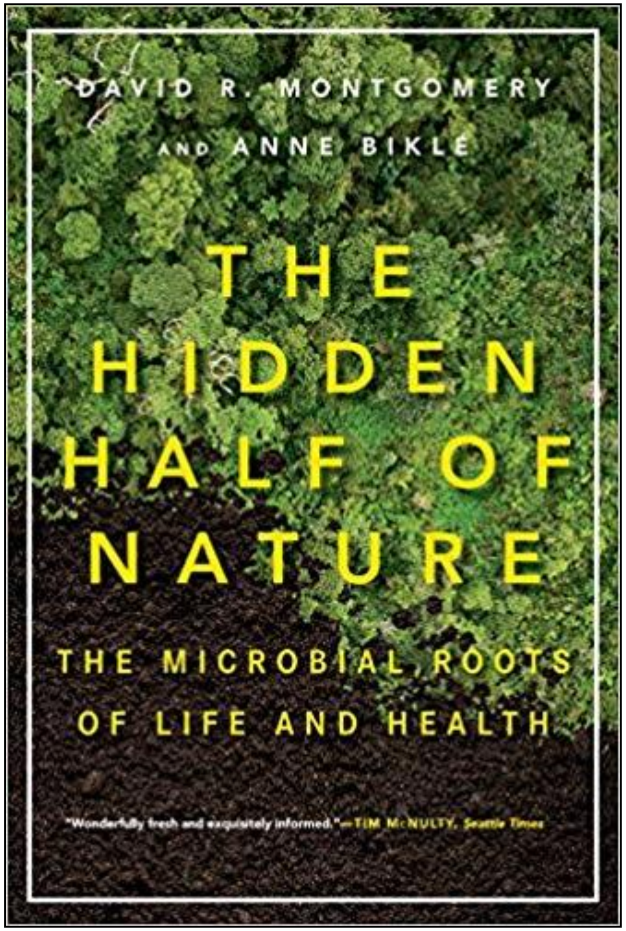
Climate Change and Microbial Ecology: Current Research and Future Trends

- **Climate Change and Microbial Ecology: Current Research and Future Trends**
- Juergen Marxsen
- Publisher: Caister Academic Press;
- April 5, 2016;
- 214 pages.



The Hidden Half of Nature: The Microbial Roots of Life and Health

- **The Hidden Half of Nature: The Microbial Roots of Life and Health**
- David R. Montgomery and Anne Biklé
- Publisher: W. W. Norton and Company
- 1 edition
- 2016
- 320 pages.

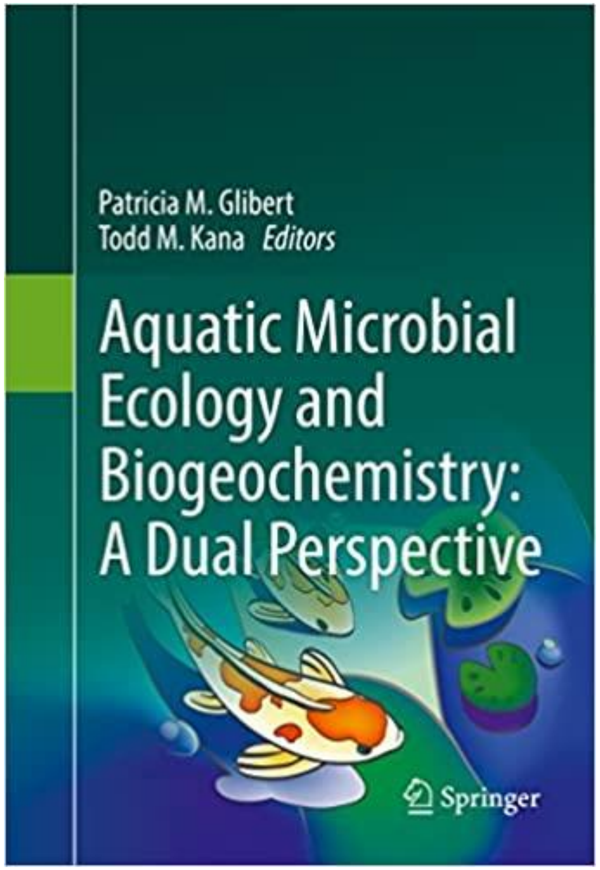


Books

Microbial Ecology

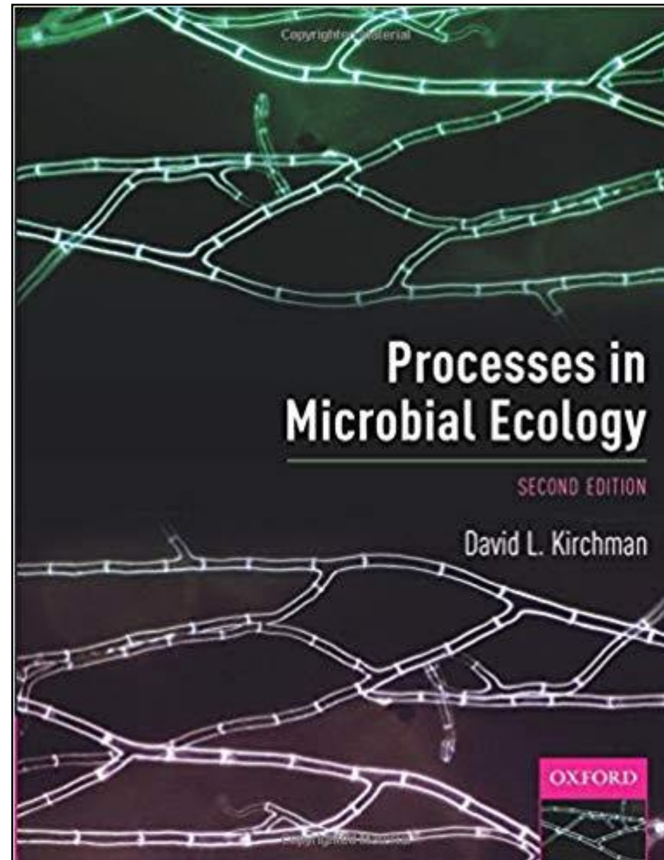
Aquatic Microbial Ecology and Biogeochemistry: A Dual Perspective

- **Aquatic Microbial Ecology and Biogeochemistry: A Dual Perspective.**
- Editors: P. M., Glibert and T. M., Kana.
- Springer
- 2016
- 491 pages.



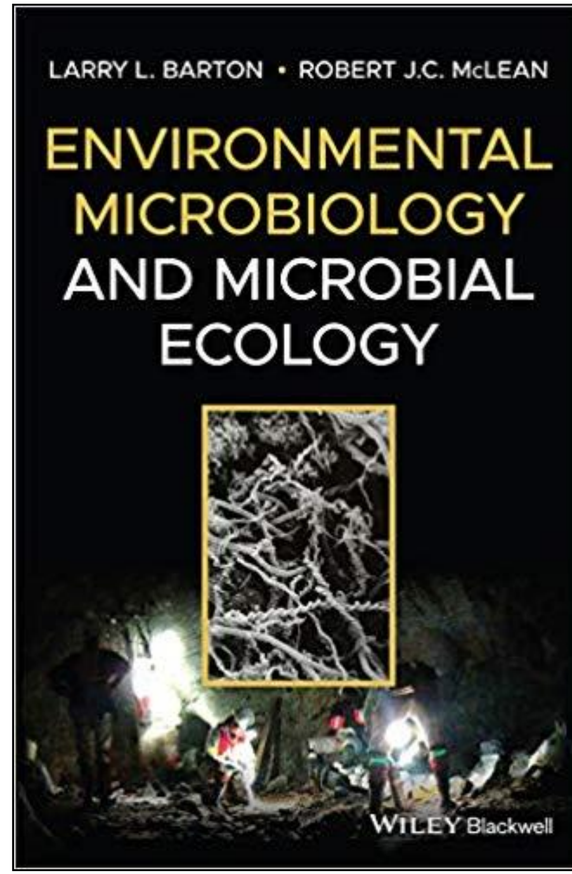
Processes in Microbial Ecology

- **Processes in Microbial Ecology**
- David L. Kirchman
- Publisher: Oxford University Press;
- 2 edition
- 2018
- 336 pages.



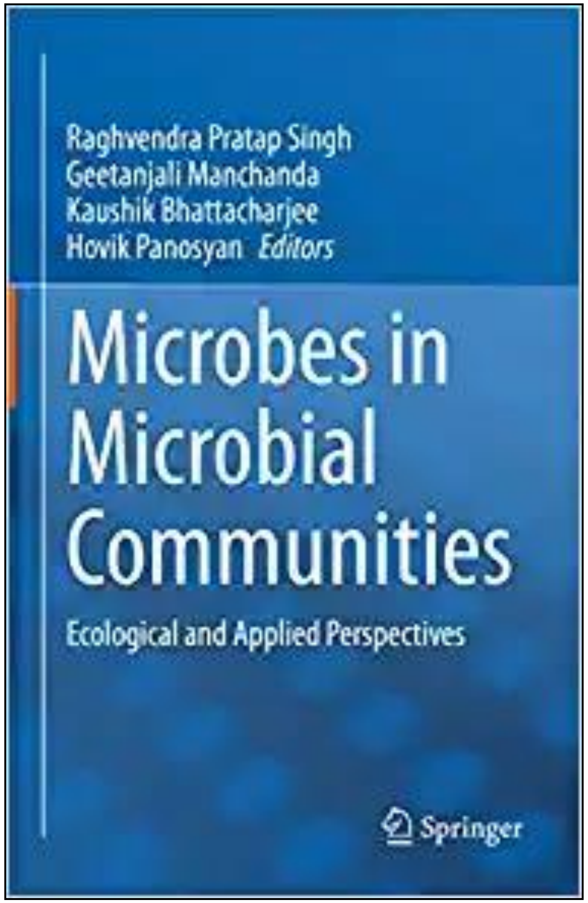
Environmental Microbiology and Microbial Ecology

- **Environmental Microbiology and Microbial Ecology**
- Larry L. Barton and R.J.C. McLean
- Wiley-Blackwell;
- 2019
- 464 pages.



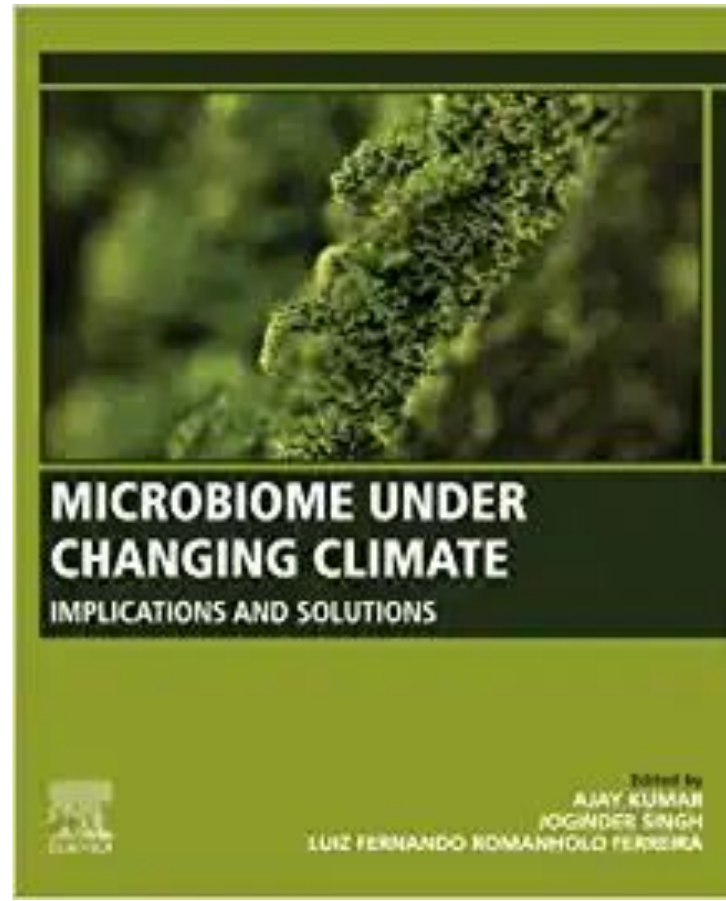
Microbes in Microbial Communities: Ecological and Applied Perspectives

- **Microbes in Microbial Communities: Ecological and Applied Perspectives**
- Raghvendra Pratap Singh, Geetanjali Manchanda, et al.
- Springer
- 2021.



Microbiome Under Changing Climate: Implications and Solutions

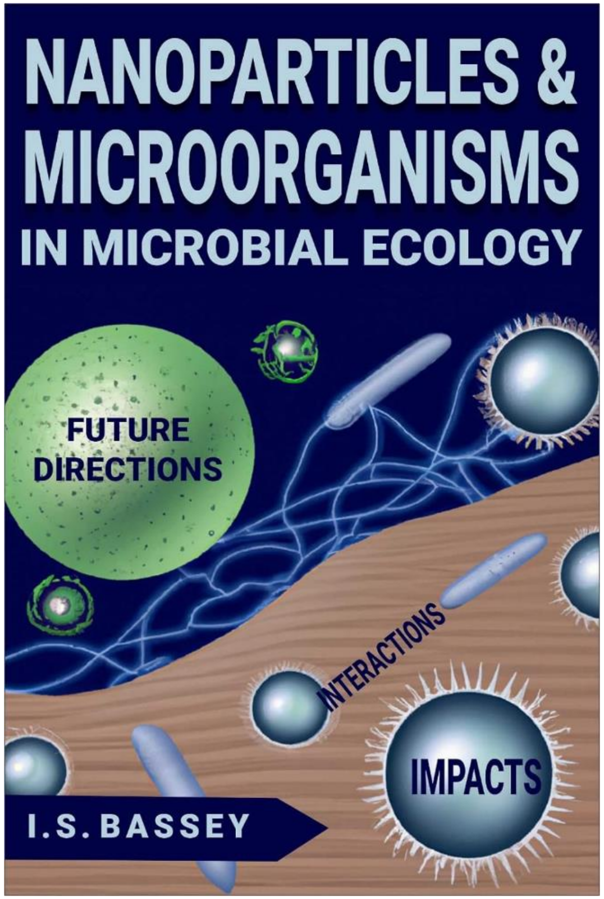
- **Microbiome Under Changing Climate: Implications and Solutions**
- Ajay Kumar, Joginder Singh, Luiz Fernando Romanholo Ferreira
- Elsevier Science
- 2022
- 574 pages



Books

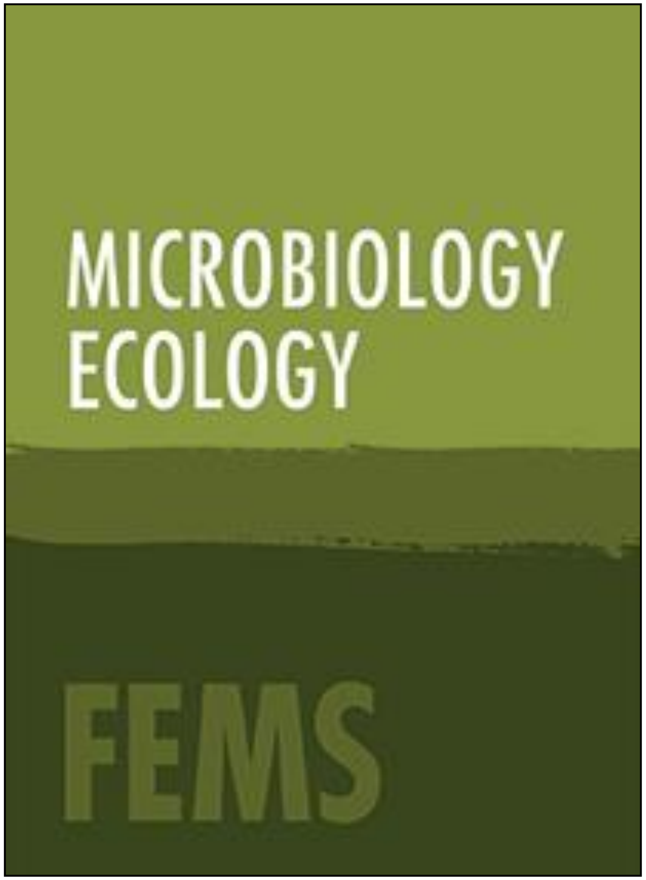
Nanoparticles and microorganisms in microbial ecology: interactions, impacts, and future directions

- **Nanoparticles and microorganisms in microbial ecology: interactions, impacts, and future directions**: Exploring the Interactions, Impacts, and Applications ... Ecology (The World of Nanoparticles Book 2)
- by I.S. Bassey
- B0BYVZXN5M
- 2023
- 52 pages



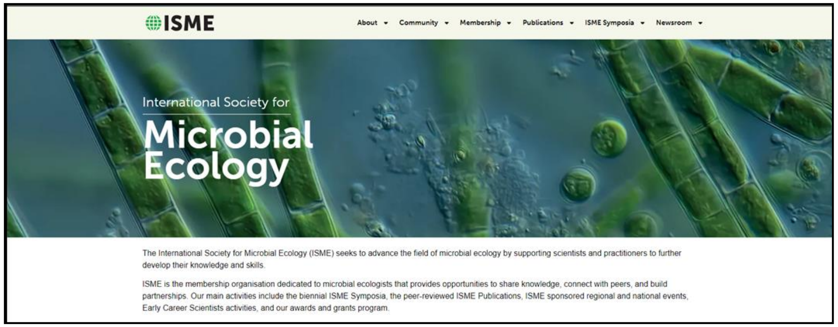
Microbial Ecology

- Microbial Ecology is a dedicated international forum for the presentation of high-quality scientific investigations of how microorganisms interact with their environment, with each other and with their hosts.



International Society for Microbial Ecology (ISME)

- The International Society for Microbial Ecology (ISME) is the principal scientific society for the burgeoning field of microbial ecology and its related disciplines.
- ISME is a non-profit association and is owner of the International Symposia on Microbial Ecology and also owner of The ISME Journal which is published by Springer Nature (impact factor 2016 9.6 - Reuters Thomson).

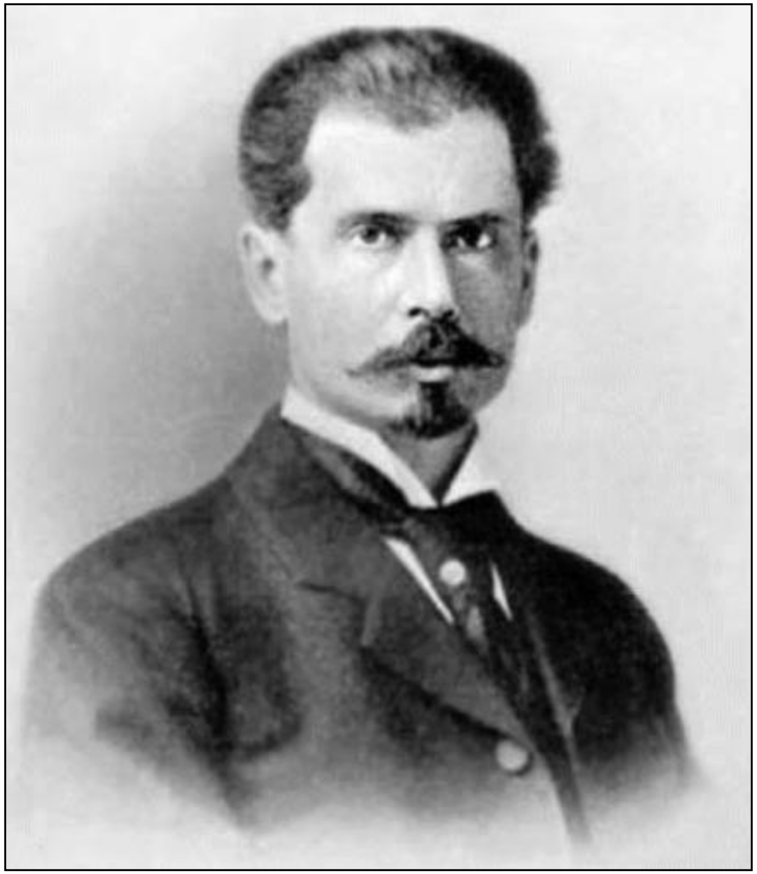


International Society for Microbial Ecology (ISME)

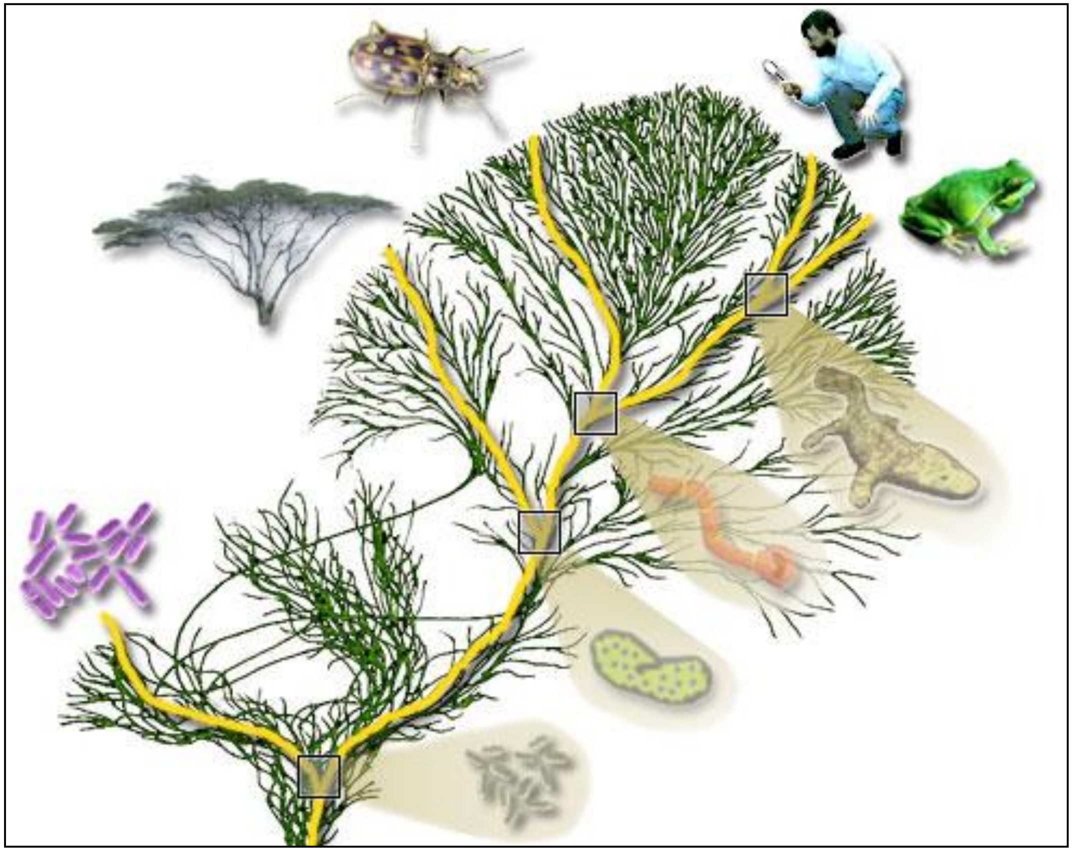
- The International Society for Microbial Ecology (ISME) seeks to advance the field of microbial ecology by supporting scientists and practitioners to further develop their knowledge and skills.
- ISME is the membership organisation dedicated to microbial ecologists that provides opportunities to share knowledge, connect with peers, and build partnerships.
- Our main activities include the biennial ISME Symposia, the peer-reviewed ISME Publications, ISME sponsored regional and national events, Early Career Scientists activities, and our awards and grants program.

Nikolaievich Winogradsky (1856-1953), the Father of Microbial Ecology

- Sergei Nikolaievich Winogradsky (1856-1953), the Father of Microbial Ecology.
- Sergei Winogradsky was born in 1856 in Russian-controlled Kiev.
- In his younger days he excelled in school, even receiving a gold medal for scholastic performance. However, during college he struggled to satisfy his curious mind.



Phylogenetic tree of life



All organisms are connected by the passage of genes along the branches of the phylogenetic Tree of Life.

Microbial ecology
Ecology definition
Old and new definition

- Branch of science dealing with the relationship of living things to their environments, coined by German zoologist Ernst Haeckel (1834-1919).
- The study of the detrimental effects of modern civilization on the environment, with a view toward their prevention or reversal through conservation.

Microbial ecology
Environmental microbiology

- Microbes are the tiniest creatures on Earth, yet despite their small size, they have a huge impact on us and on our environment.
- Microbial ecology is:

1. the study of microbes in the environment and
2. their interactions with each other.

Importance of ecology

- Ecology is not synonymous with environment, environmentalism, natural history, or environmental science.
- It is closely related to evolutionary biology, genetics, and ethology.
- An important focus for ecologists is to improve the understanding of how biodiversity affects ecological function.

Ethology:
1. The science of animal behaviour.
2. the study of human behaviour and social organization from a biological perspective.

Importance of ecology

1. Microbes and Ecosystem Niches;
2. Organization of Ecosystems;
3. Role of Microbes in Biogeochemical Cycling;
4. Microbial Environments and Microenvironments.

A Biofilm of Thermophilic Bacteria: Thermophiles, which thrive at relatively high temperatures, occupy a unique ecological niche. This image shows a colony of thermophilic bacteria at Mickey Hot Springs in Oregon, USA.

Organization of Ecosystems

- Microorganisms serve essential roles in the complex nutrient exchange system that defines an ecological community.
- An ecosystem is a unified system of exchange made up of autotrophic producers, heterotrophic consumers, and decomposers.
- Microorganisms play a vital role in every ecological community by serving as both producers and decomposers.

Trophic level: The level of an organism in a community based on its feeding requirements (e.g., producers are at the lowest trophic level).

Microbial ecology
Importance in evolution

- Due to the high level of horizontal gene transfer among microbial communities, microbial ecology is also of importance to studies of evolution.

Wikipedia,2016

Microbial ecology
Environmental microbiology

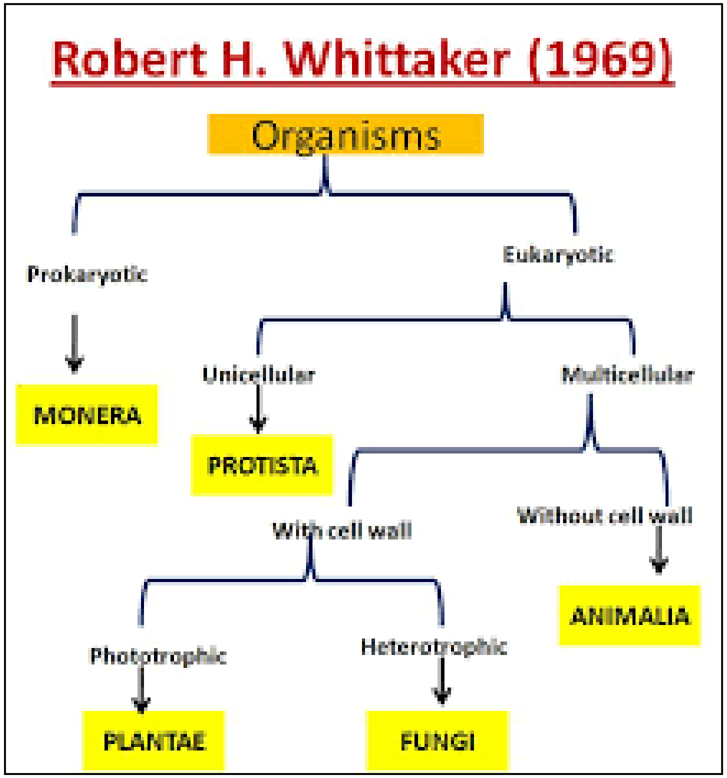
- Microorganisms or microbes are microscopic organisms that exist as unicellular, multicellular, or cell clusters.
- They can be divided into six major types:

1. bacteria,
2. archaea,
3. fungi,
4. protozoa,
5. algae, and
6. viruses.

The second natural classification scheme
The five-kingdom system, proposed in 1969 by Whittaker

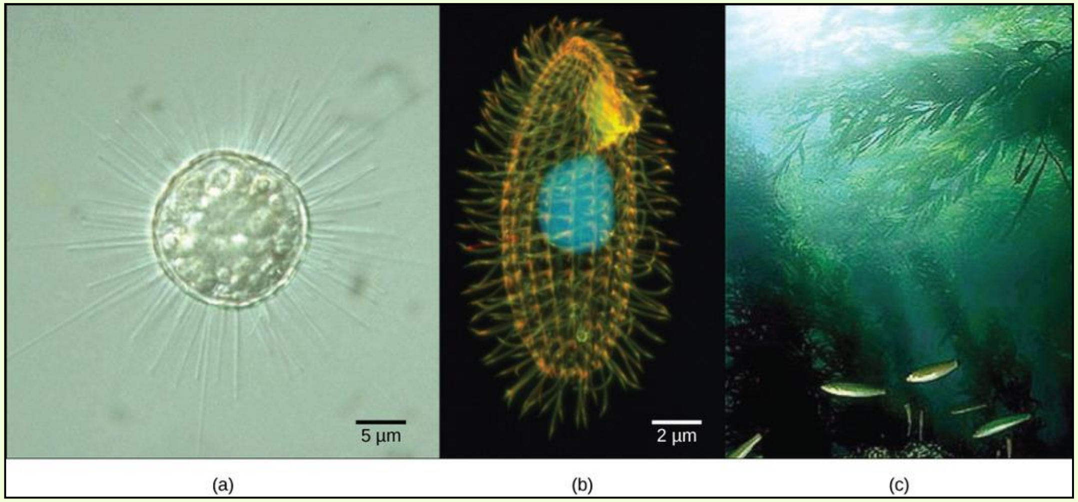
- Phylogenetic and symbiogenetic tree of living organisms, showing the origins of eukaryotes & prokaryotes:

1. Animalia,
2. Plantae,
3. Fungi,
4. Protista, and
5. Monera.



The five-kingdom system, proposed in 1969 by Whittaker

- Protist varieties:
- Protists range from the microscopic, single-celled (a) Acanthocystis turfacea and the (b) ciliate Tetrahymena thermophila, both visualized here using light microscopy, to the enormous, multicellular (c) kelps (Chromalveolata) that extend for hundreds of feet in underwater "forests.



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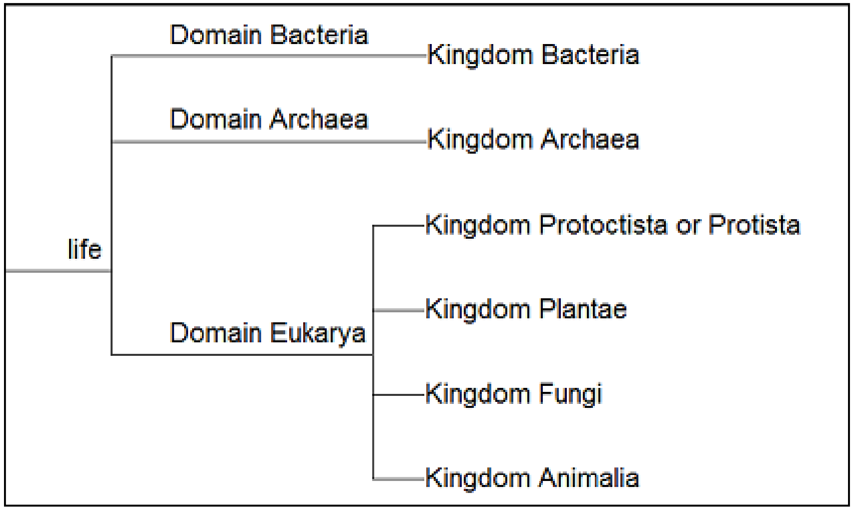
The second natural classification scheme
Six kingdoms
Proposed by Woese *et al.*,1977

1. Kingdom Eubacteria
2. Kingdom Archaebacteria
3. Kingdom Protoctista
4. Kingdom Plantae
5. Kingdom Fungi
6. Kingdom Animalia



Based on this work, they concluded that the Archaea are more closely related to humans than to bacteria.

Kingdom Animalia or animals

Examples:

Arthropoda – includes insects, arachnids, and crustaceans

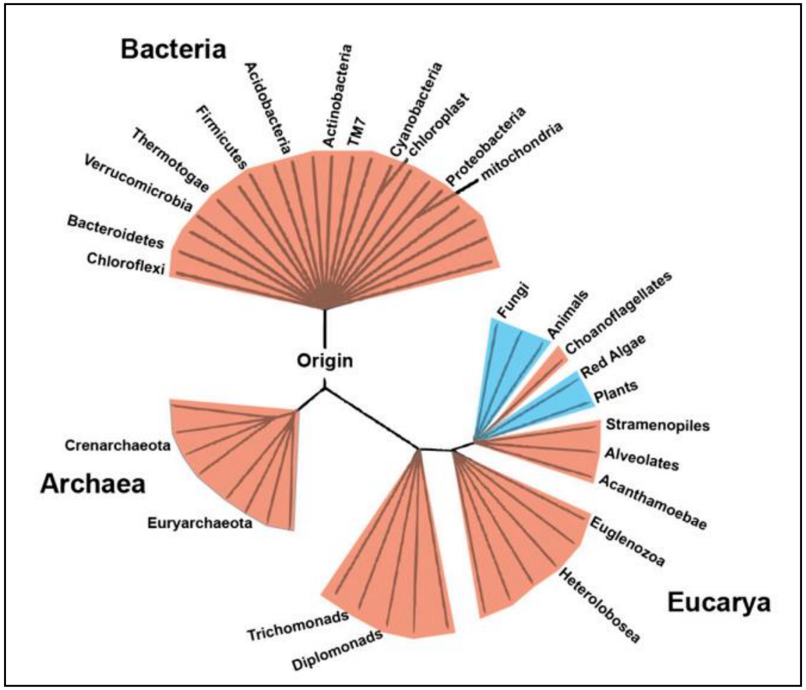
Chordata – includes vertebrates and, as such, human beings.

Microbial ecology

Bacteria and archaea are two microbial domains in life tree

Proposed by Woese *et al*.,1977

- A version of the universal tree of life based on rRNA sequence analysis, adapted from Pace (2009), with multicellular lineages indicated in blue and those lineages that are primarily unicellular highlighted in red.



Fierer and Lennon,2011

The Third natural classification scheme
Six kingdoms
Proposed by T. Cavalier-Smith,1998

- Thomas Cavalier-Smith,1998 has published a six-kingdom model on the evolution and classification of life, particularly protists.

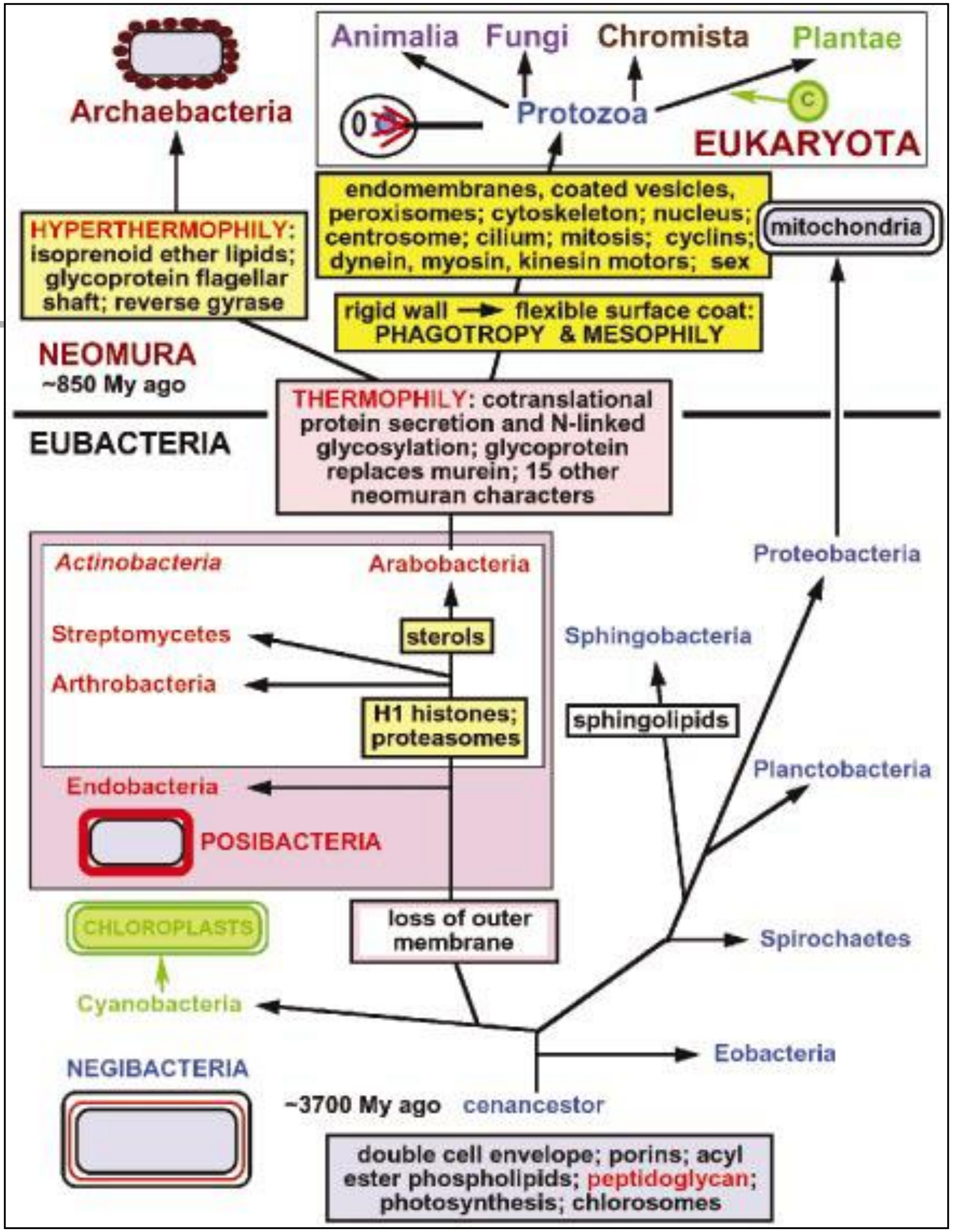
1. Animalia
2. Protozoa
3. Fungi
4. Plantae (including red and green algae),
5. Chromista
6. Bacteria

- This was revised in subsequent papers.
- In total, his views have been influential but controversial, and not always widely accepted.

Wikipedia,2011;..

The bacterial origins of eukaryotes as a two-stage process.

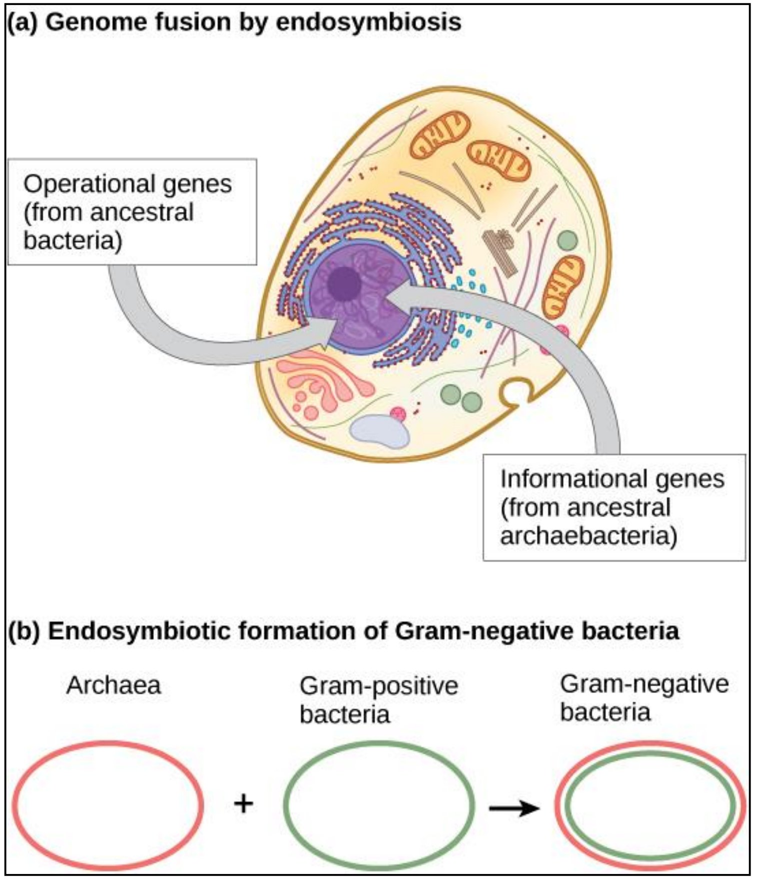
This paper very strongly supports actinobacterial origin of neomura.



Cavalier-Smith,2001

Endosymbiosis in eukaryotes

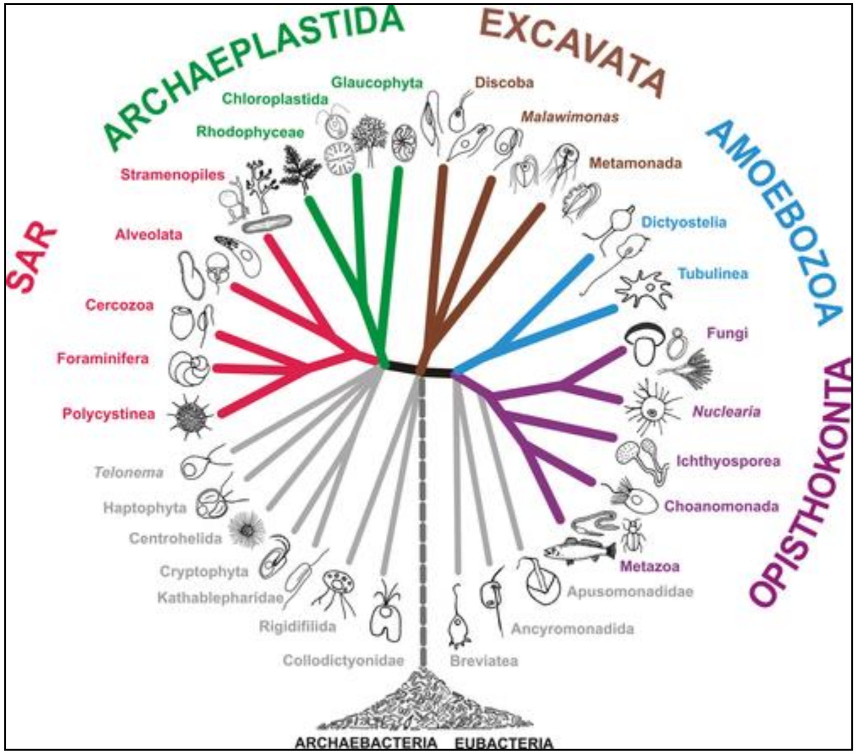
- The theory that mitochondria and chloroplasts are endosymbiotic in origin is now widely accepted.
- More controversial is the proposal that (a) the eukaryotic nucleus resulted from the fusion of archaeal and bacterial genomes; and that (b) Gram-negative bacteria, which have two membranes, resulted from the fusion of Archaea and Gram-positive bacteria, each of which has a single membrane.



Boundless.com

The revised classification of eukaryotes

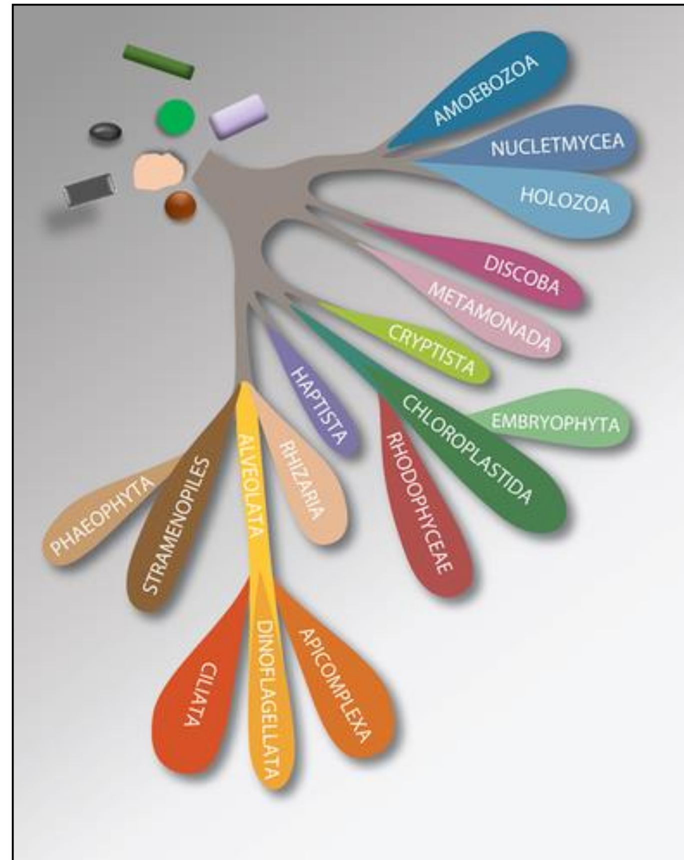
- Plants (Plantae) are placed in Archaeplastida.
- The glaucophytes may be original algal type that led to green plants and red algae.



Adl *et al.*,2012

The revised classification of eukaryotes

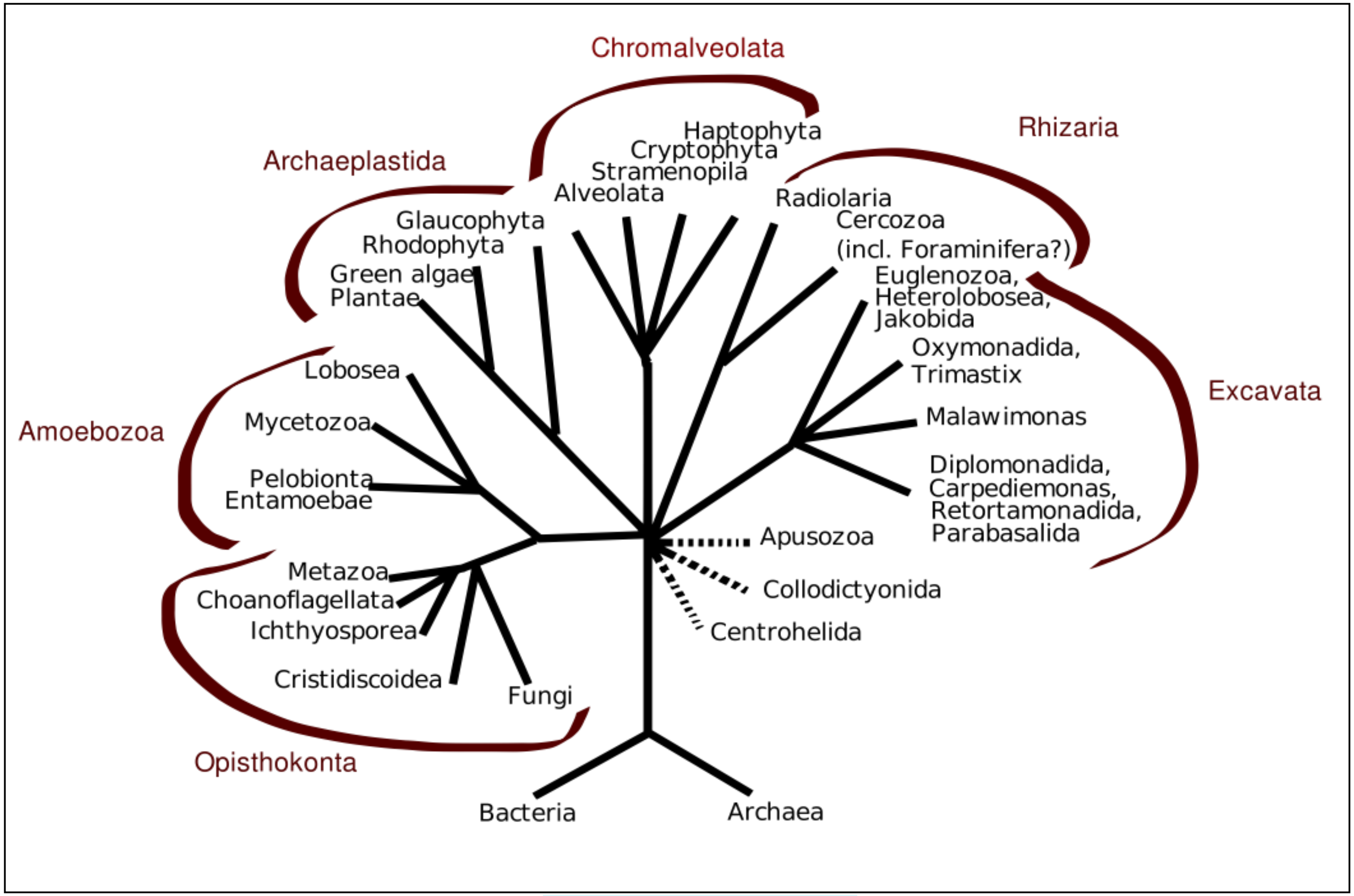
- Overview of the diversity of protists among eukaryotes. Amoebozoa, Nucletmycea, and Holozoa together form the Amorphea;
- The Diaphoretickes includes Crypista, Chloroplastida and Embryophyta, Rhodophyceae, Haptista, Rhizaria, Alveolata (Apicomplexa, Dinoflagellata, Ciliata), Stramenopiles and Phaeophyta.



Adl *et al.*,2018

The revised classification of eukaryotes

The Six Kingdoms:This is one hypothesis of eukaryotic relationships. The Opisthokonta group includes both animals (Metazoa) and fungi. Plants (Plantae) are placed in Archaeplastida



Boundless.com

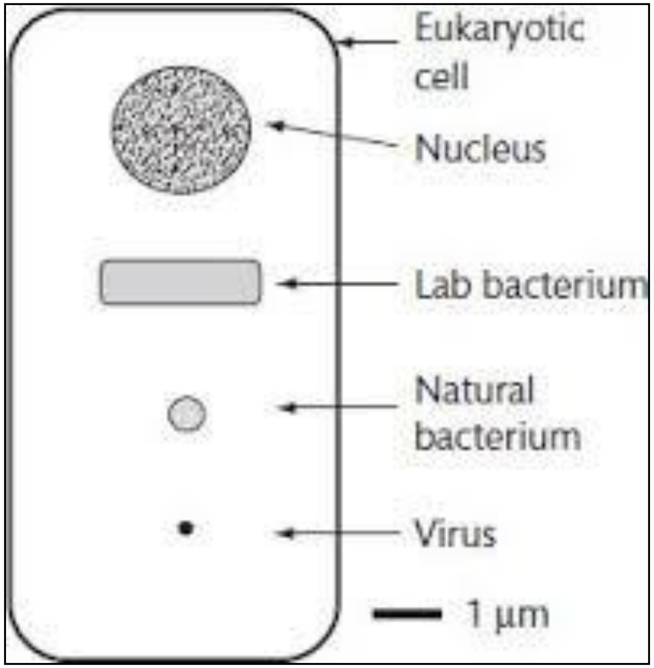
Archaea

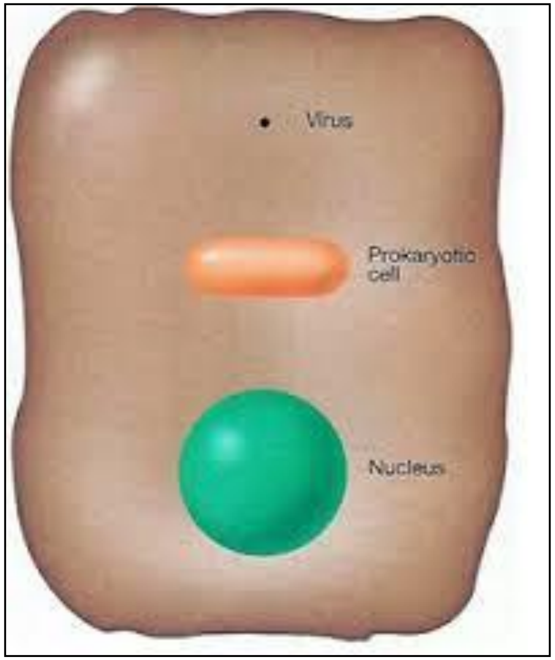
- Most of the archaea are methanogens and extremophilic in origin.
- They reside in extremely hostile conditions.

Hostility	Name
40-85°C	Thermophile
>85°C	Hyperthermophiles
20-40°C	Mesophiles
<20°C	Psychrophiles
15% of NaCl	Halophiles
pH>7	Alkaliphiles
pH<7	Acidophiles

Microbial ecology

Diversity in cell size

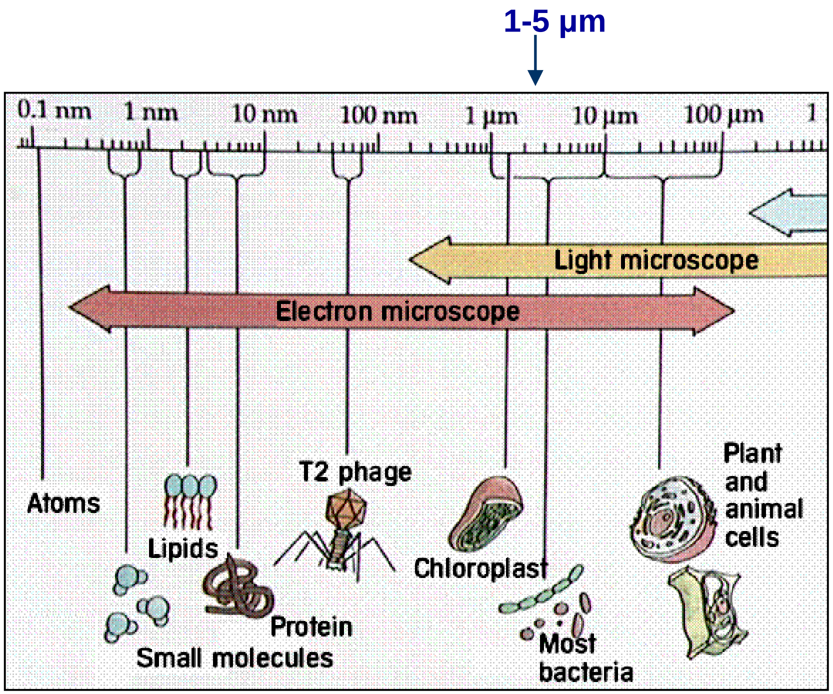




Rightler;..

Microbial ecology
Diversity in cell size

- Average size of prokaryotic cells: 0.2-2.0 µm in diameter x 1-10 µm in length.
- Typical eukaryote 10-500 µm in length (0.01-0.5 mm).
- Bacterial cells are about one-tenth the size of eukaryotic cells.



Farabee,2007;..

Diversity in cell size

Exceptional

Epulopiscium fishelsonii is a giant procaryotes

- The prokaryotic cell diameters are typically near 1 μm, whereas eukaryotic cells are 10 μm or larger is generally true, but significant exceptions exist.
- *Epulopiscium fishelsonii* , as a giant procaryote found in intestinal symbionts of certain species of surgeon fish in the Red Sea.
- Cigar shaped cells with a diameter as great as 80 μm and lengths up to 600 μm.

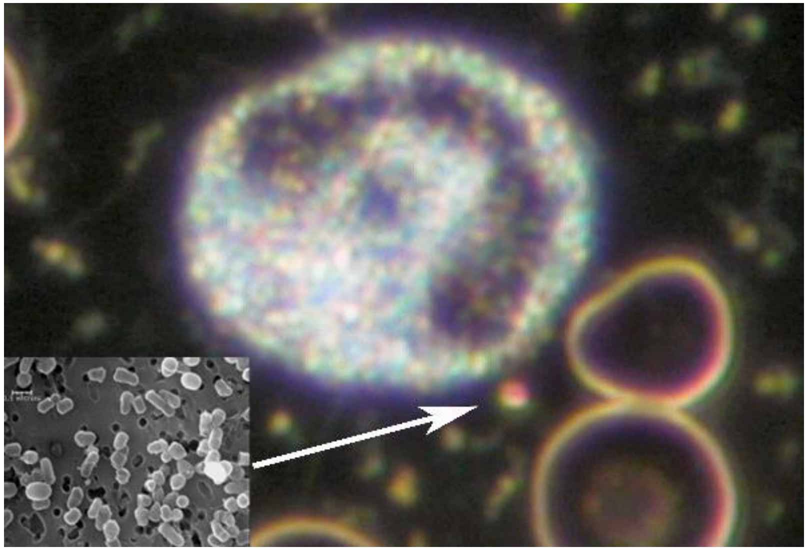
The Prokaryotes (chapter 1.7),2006;..

Diversity in cell size

Exceptional

Nanobacteria are the smallest cell-walled organisms on earth

- Nanobacteria (singular nanobacterium) or nanobes are nano-sized bacteria found in organisms (even human blood) and rocks.
- Smallest cell-walled organisms on earth, smaller than 300 nm (1/10 the size of bacteria).
- Nanobacteria self-replicate and form colonies even though they range from 50 to 200 nm in size.



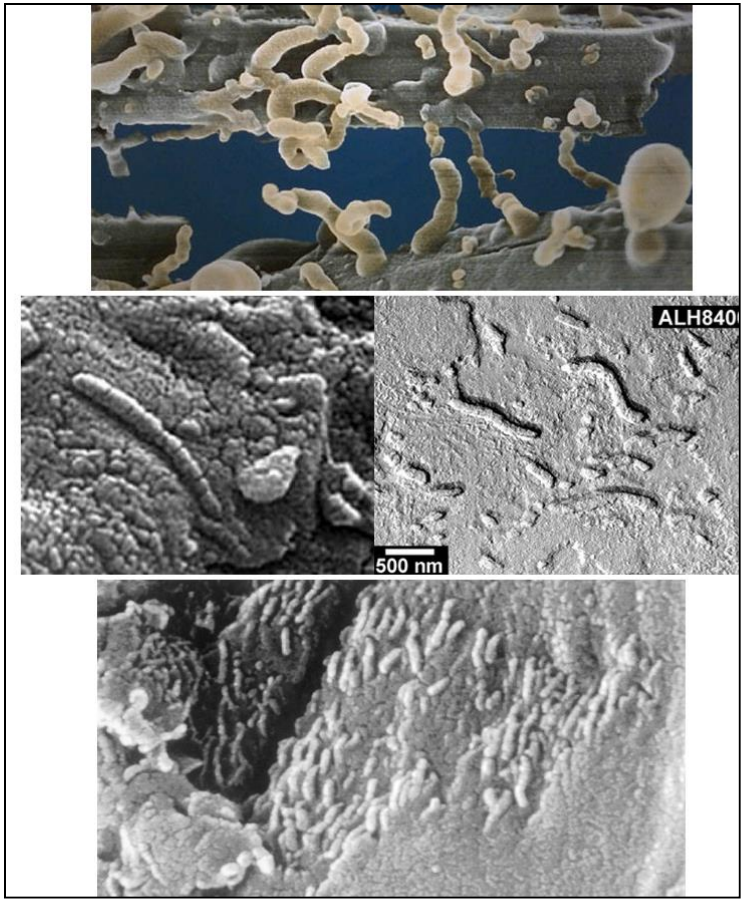
Nanobacteria compared to bacteria in size. Microfossils of Martian Nanobacteria discovered in Martian Meteorite ALH 84001.

Joseph and Schild,2010

Diversity in cell size

Nanobacteria are the smallest cell-walled organisms on earth

- Some questioning whether or not an organism of this size has enough room to house necessary cell components such as DNA, RNA, and plasmids.
- Since they not only replicate but grow in size, it would be just one small step to cross the (arbitrary) threshold of 200 nm, to become large enough to incorporate DNA.

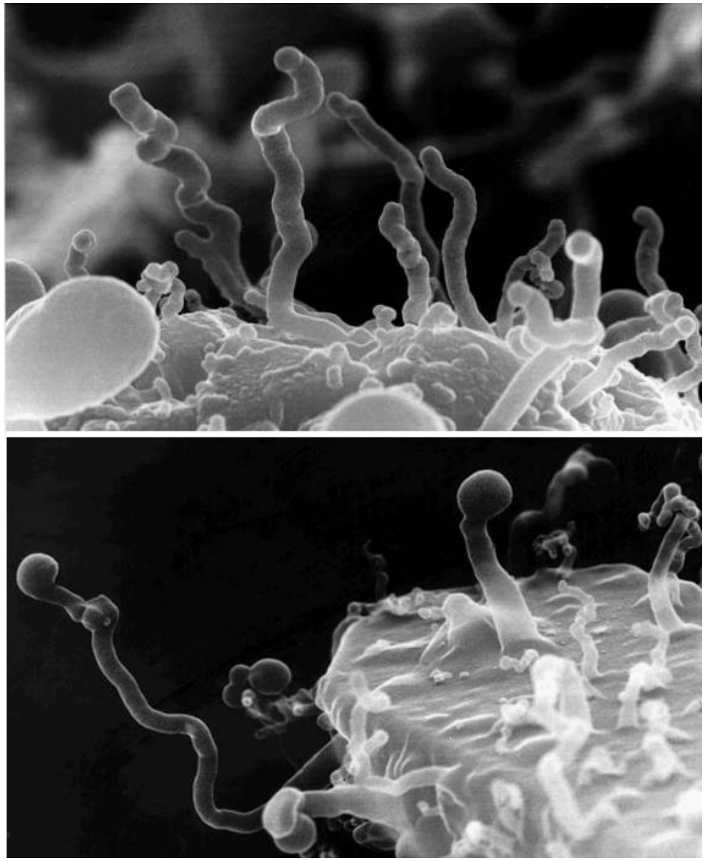


Wikipedia,2013;M.Bruckner;Joseph and Schild,2010

Diversity in cell size

Nanobacteria are the smallest cell-walled organisms on earth

- Nanobe studies challenge our perception of life.
- Microbes have already expanded our understanding of the harsh conditions that can support life.
- So, if nanobes do exist as living biota, they will broaden our perspective on the scale of life.
- Potential role in forming kidney stones.



Wikipedia,2013;M.Bruckner;Joseph and Schild,2010

Microbial ecology

Environmental microbiology

- Microorganisms, by their omnipresence, impact the entire biosphere.
- Microbial life plays a primary role in regulating biogeochemical systems in virtually all of our planet's environments including:

1. some of the most extreme, from frozen environments and acidic lakes, to
2. hydrothermal vents at the bottom of deepest oceans, and
3. some of the most familiar, such as the human small intestine.

Wikipedia,2016

Microbial ecology

Cell concentrations (conc.) and numbers of bacterial species from a wide range of environments

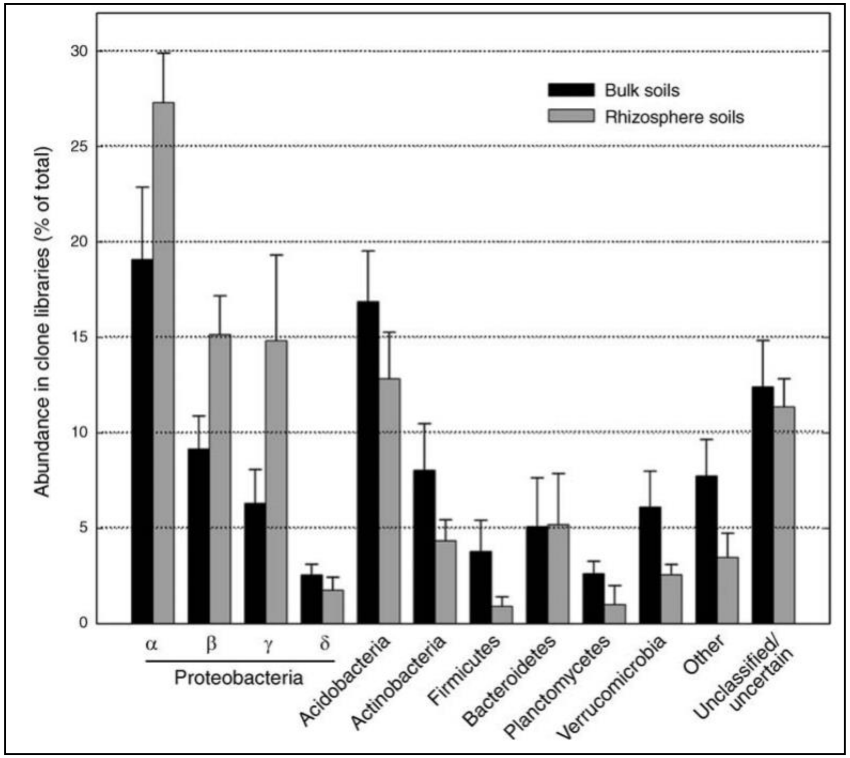
Habitat	Cell conc. (Source)	Mean no. per sample (Source)	Total volume of sample
Soil	10^{10} cells/cm^3	10000	1 cm^3
Air	10^6 cells/m^3	100	2.5-5.4 m^3
Human skin	10^7 cells/cm^2	200	70 cm^2
Human gut	10^{12} cells/g feces	1000	0.2 g
Human mouth (saliva)	10^8 cells/mL saliva	5700	10 mL
Marine water	10^5 to 10^6 cells/mL sea water	4000	1000 mL
Marine hydrothermal vent fluids	10^5 cells/mL	260	3 L
Glacial ice	10^6 cell/mL	100	100 mL
Sewage	10^9 cells/mL	2500	100 mL
Anoxic subglacial lake	10^6 cells/mL	70	250 mL
Baltic Sea (brackish surface water)	10^6 cells/mL	1500	5000 mL
Leaf surfaces	10^5 cells/cm^2	250	5000 cm^2

Fierer and Lennon,2011

Microbial Ecology

Ecological classification of soil bacteria

- The relative abundances (mean + standard error, SE) of different bacterial phyla in soil as estimated from a meta-analysis of published clone library results reported for rhizosphere and bulk soil samples.
- A total of 32 libraries were included in the analysis:
- 16 from bulk soils, and
- 16 from rhizosphere soils.



Fierer *et al.*,2007

Microbial Ecology
Key terms

- Allochthonous (transient) microbes: Originating in a place other than where it is found. Allochthonous microorganisms get energy from outside sources. Most pathogens are allochthonous microorganisms.
- Amensalism: association between organisms of two different species in which one is inhibited or destroyed and the other is unaffected. The black walnut tree is an example of amensalism. It releases a chemical from its roots that harms the plants around it.
- Autochthonous (resident) microbes: indigenous rather than descended from migrants or colonists. Autochthonous microorganisms are primary producers (create own energy, for example, through photosynthesis.

Microbial Ecology

Key terms

- Autotroph: Any organism that can synthesize its food from inorganic substances, using heat or light as a source of energy.
- Biogeochemistry: The scientific study of biological, geological, and chemical processes in the natural environment and especially of their mutual relationships.
- Microbiome: is the collection of all microbes, such as bacteria, fungi, viruses, and their genes, that naturally live on our bodies and inside us.
- Microbiota: consists of all living members forming the microbiome. As phages, viruses, plasmids, prions, viroids, and free DNA are usually not considered as living microorganisms, they do not belong to the microbiota.

The microbiome
Microbiota

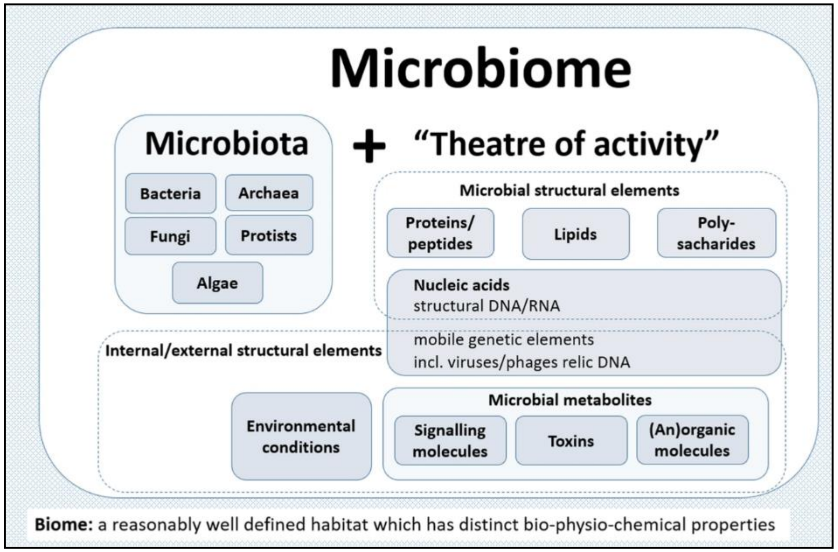
- Microbiota is very different from microbiome.
- Microbiome is MORE than microbiota alone.
- To put it simply, if microbiome is a house, the microbiota is the people who live there.
- Microbiome refers to the entire habitat. The term includes microorganisms such as bacteria and eukaryotes as well the genes that comprise them and the environmental factors that influence them.
- Microbiota is the unique combination of microorganisms that exist in a specific environment. This may include bacteria, archaea, or eukaryotes.

Allucent,2024

The microbiome
Microbiota

- The term microbiome encompasses both:

1. the microbiota (community of microorganisms), and
2. their theatre of activity (structural elements, metabolites/signal molecules, and the surrounding environmental conditions.



Wikipedia,2024

Microbial consortia

Microbial community

- Microbial consortia or microbial community: is a group of diverse microorganisms that have the ability to act together in a community.
- Consortiums can be endosymbiotic or ectosymbiotic, or occasionally may be both.
- E.g.
- A group of two or more symbiotic microorganisms capable of surviving under diverse conditions by the formation of synergistic population structures such as stromatolites, microbial mats, biofilms, etc.

Microbial ecology

Biogeochemical cycling

- Microbial communities play a central role in virtually every biogeochemical cycle on earth, driving global carbon and nutrient cycling with direct feedback on ecosystem functioning and productivity.
- A multitude of microorganisms also associate with higher organisms and collectively function as a microbiome.
- It is now well established that every higher organism investigated, from plants, insects, and fish up to mice, apes, and humans, harbors a microbiome.

Wikipedia,2016

Microbial ecology

Biogeochemical cycling

- Findings in the last decade revealed that these microbial communities do not simply inhabit our skin or intestine but also appear to influence processes including behavior, appetite, and health.

Mammals*	Other Animals*	Plants*
Human	Zebrafish	Barley
Elephant	*Hyrda*	Maize
Chimpanzee, gorilla	Termites	*Arabidopsis* (Thale cress)
Rabbit	Cockroaches	Grapevine
Polar Bear	Springtails	Rice
Horse	Thrips	Hairy bittercress

*Adapted from [3–5]. Note that many studies investigating microbiomes focused on specific taxonomic groups (e.g., prokaryotes), and other important domains (e.g., fungi or protozoa) have often not been assessed.

doi:10.1371/journal.pbio.1002378.t001

Marcel *et al.*,2016

Microbial Ecology
Key terms

- Bioremediation: The use of biological organisms, usually microorganisms, to remove contaminants, especially from polluted water.
- Biosphere: The part of the Earth and its atmosphere capable of supporting life.
- Community: The populations of different species that exist in the same defined area (groups of populations living together as an association). Biofilms are complex communities of microorganisms attached to surfaces or associated with interfaces.
- Extremophiles: a class of organism that thrives under extreme conditions of temperature, salinity, and so on; commercially important as a source of enzymes that operate under similar conditions.

Microbial Ecology

Key terms

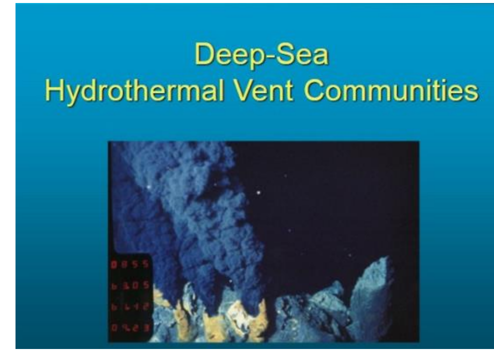
- Climax: The highest or most intense point in the development or resolution of something.
- Climax community: When the population reaches equilibrium and the communities stop changing, the system has reached a climax (activated sludge). Some ecosystems never reach a climax.
- Commensalism: The word "commensalism" is derived from the word "commensal", meaning "eating at the same table" in human social interaction. In ecology, is a class of relationships between two organisms where one organism benefits from the other without affecting it.

Microbial Ecology
Key terms

- Ecological community: A ecological community in the final stage of succession, in which the species composition remains relatively stable until a disturbance such as fire occurs(see also the term succession).

Microbial Ecology

Key terms

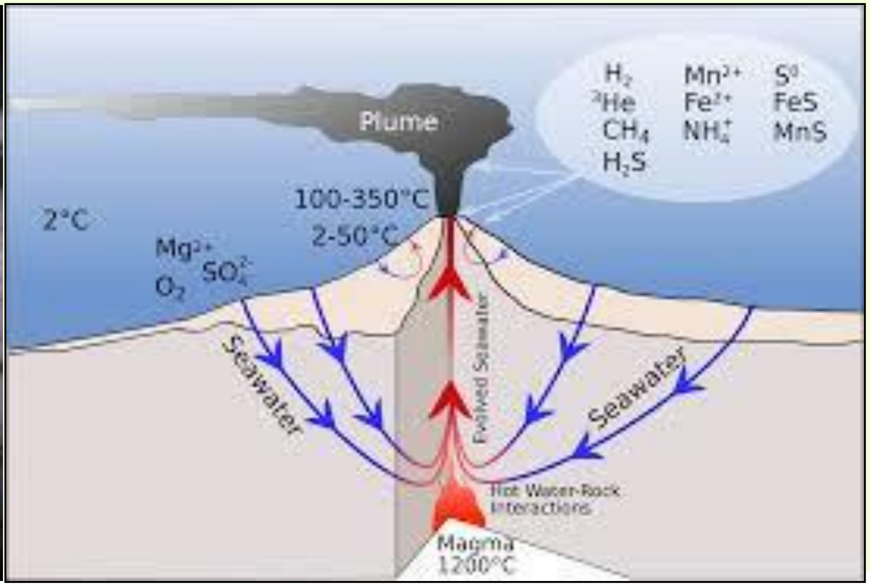


- Hydrothermal vent (deep sea ecology): Until recently, all life on Earth was believed to be dependent on the sun. But in the last 30 years, several new deep-sea ecosystems have been discovered that utilize an alternative source of energy.
- Hydrothermal vent: An opening in the sea floor out of which heated mineral-rich water flows.
- Deep Sea Vent Communities: Many archaea and bacteria live near hydrothermal vents, which are underwater. They support giant tube worms, clams and shrimp, and many other eukaryotes.

Microbial Ecology

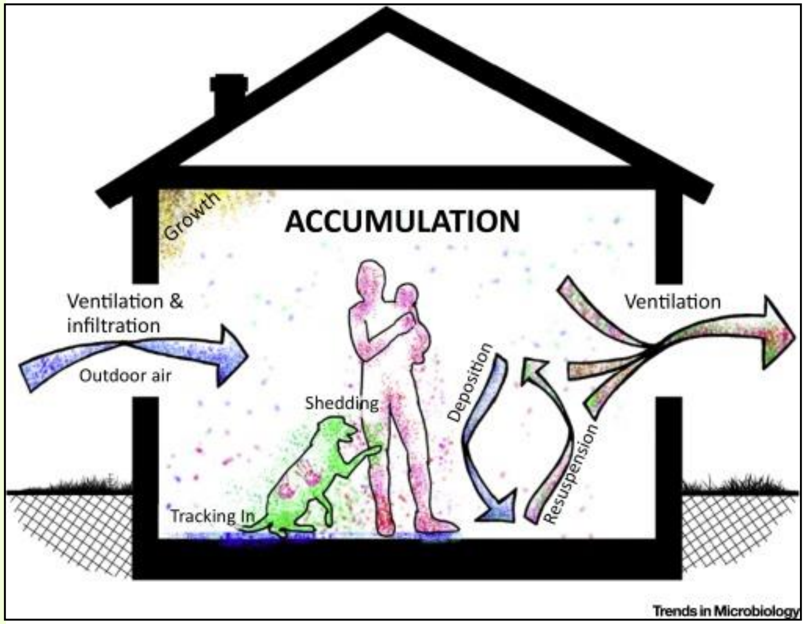
Key terms

- Hydrothermal vent: An opening in the sea floor out of which heated mineral-rich water flows.
- Plume: something (such as smoke, steam, or water).



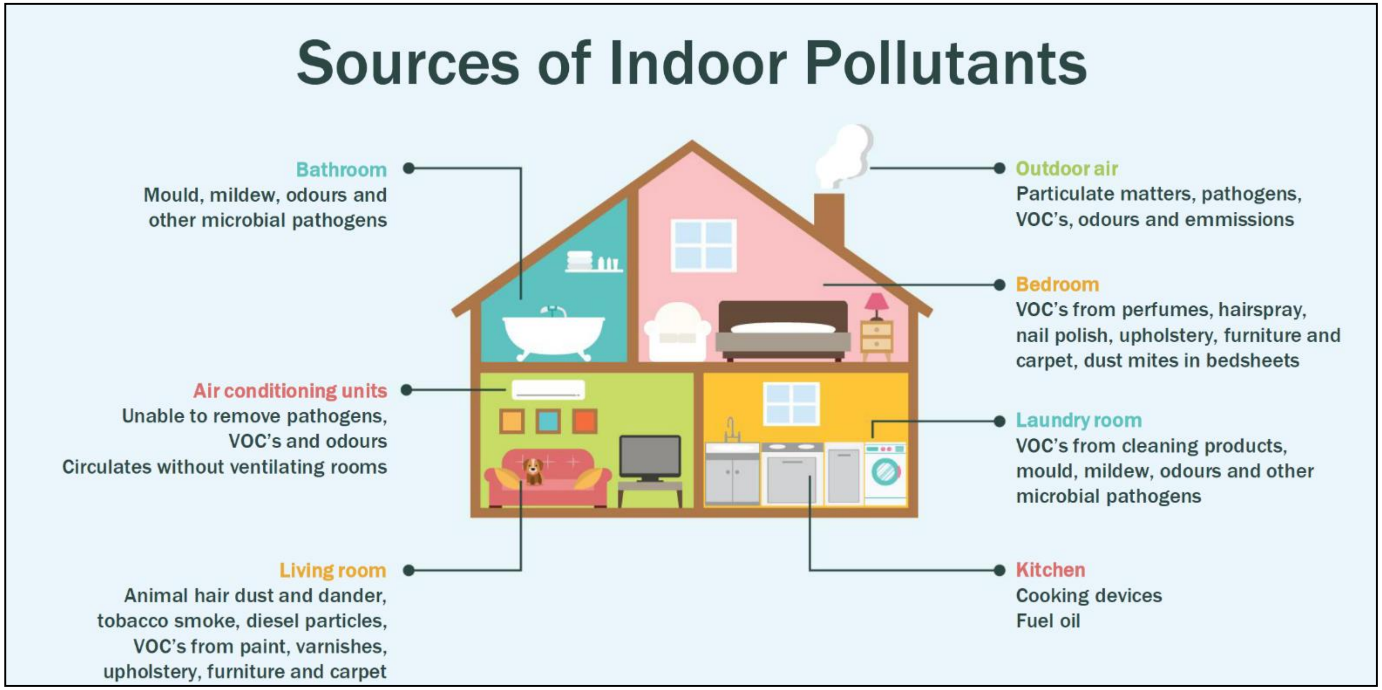
Microbial Ecology
Key terms

- Indoor: Carried on within door, inside of a house or other buildings.
- Indoor environmental quality (IEQ): refers to the quality of a building's environment in relation to the health and wellbeing of those who occupy space within it.
- IEQ is determined by many factors, including lighting, air quality, and damp conditions.



Microbial Ecology
Microflora of air
Key terms

- Indoor Air Quality Infographic showing what's in indoor air, how air quality affects our health and home, and how air purifiers can help improve air quality.



http://www.andatech.com.au

Microbial Ecology

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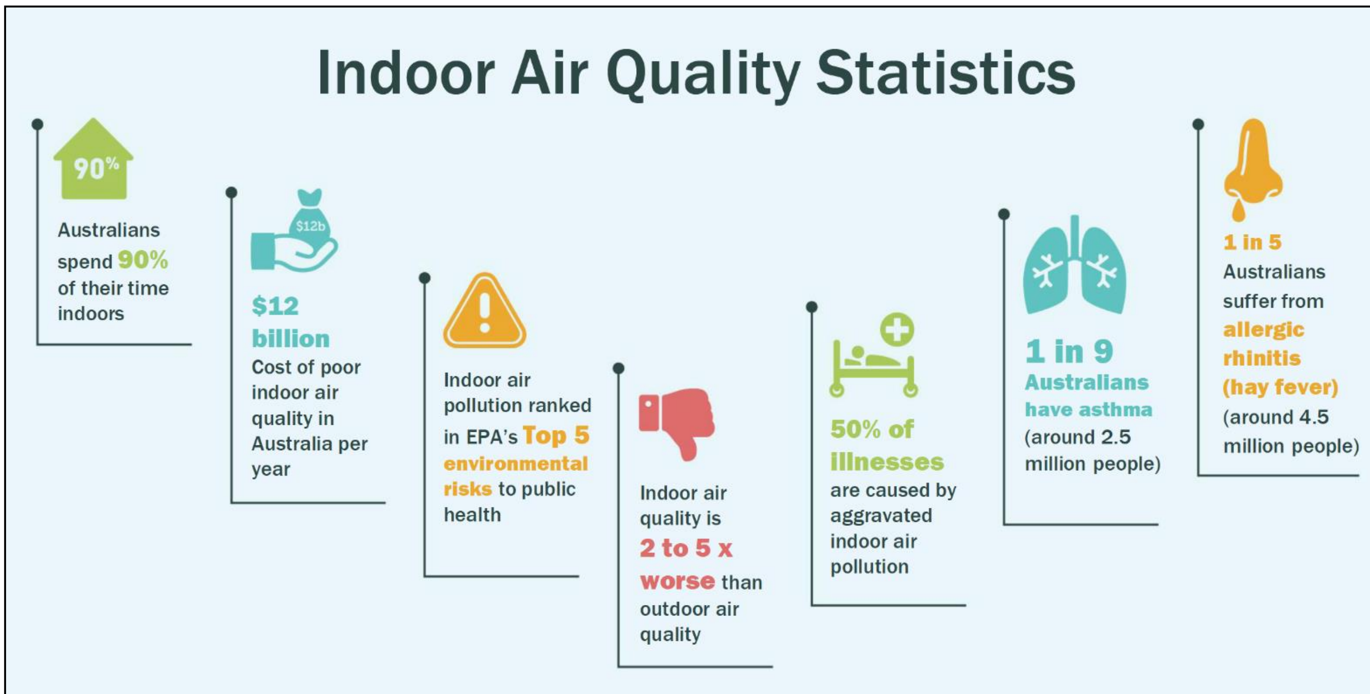

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http://www.andatech.com.au

Microbial Ecology

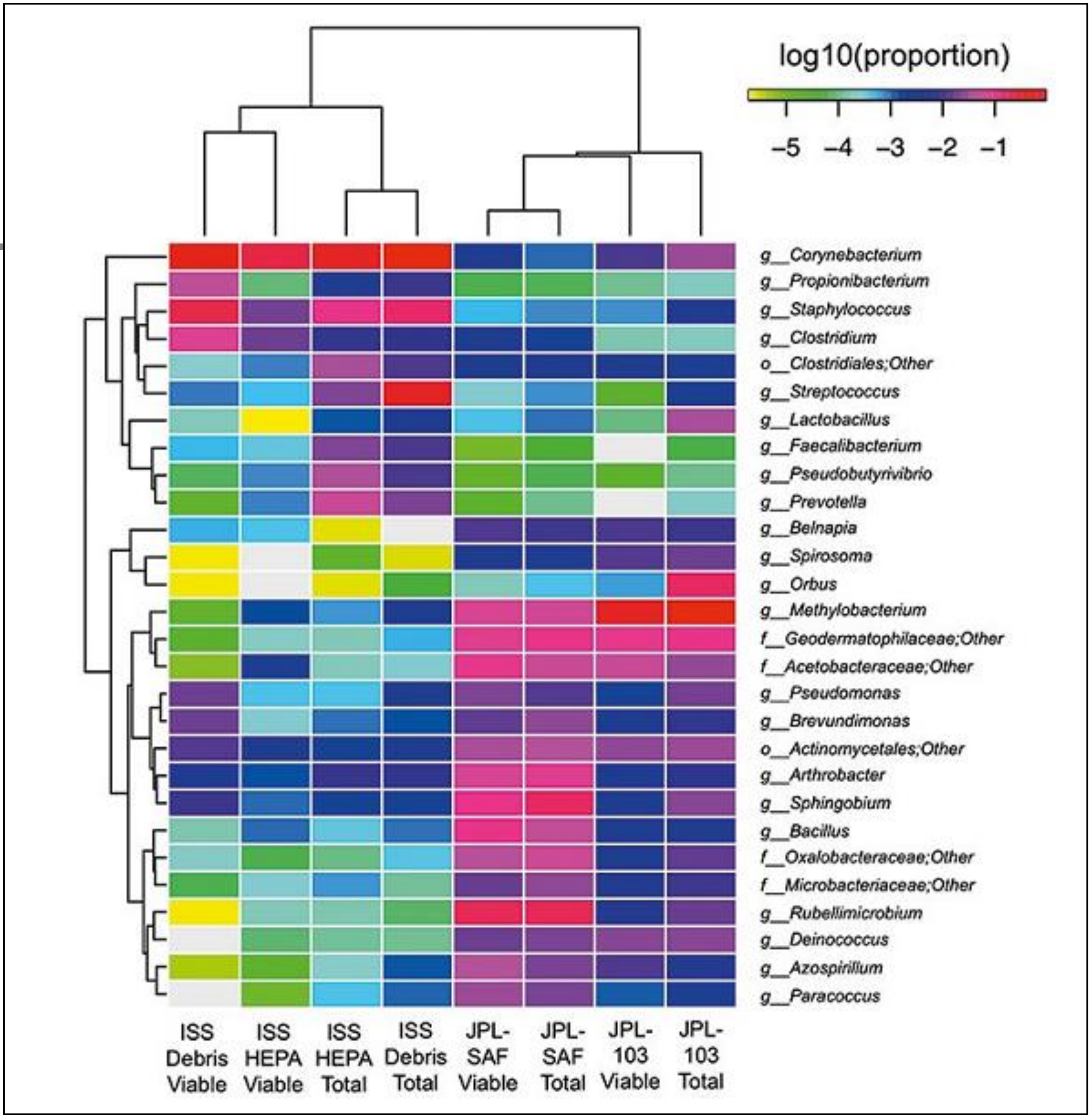
Microflora of International Space Station (ISS)

Built environment

- There's a little known, dirty story about the Internatio nal Space Station (ISS):
- It's filled with bacteria and fungi.
- A new study has found compelling evidence that microorganisms from human skin are present throughout the station, and some of the bugs could cause serious harm to as tronauts.

Science- AAAS News.htm

The international space station is home to potentially dangerous bacteria.



Science- AAAS News.htm

Microbial ecology
Microflora of water

- Water is a natural medium of a habitation of various microorganisms. In sweet and salty waters representatives of all groups of bacteria are found Protozoa, Fungi, Viruses, Water - plant.
- The collection of all aqueous microorganisms is named as a microbial plankton.
- The microflora of reservoirs consists of two groups of microbes:

1. **Autochtonal** (or aqueous) microorganisms, and
2. **Allochtonal** (brought from the outside).

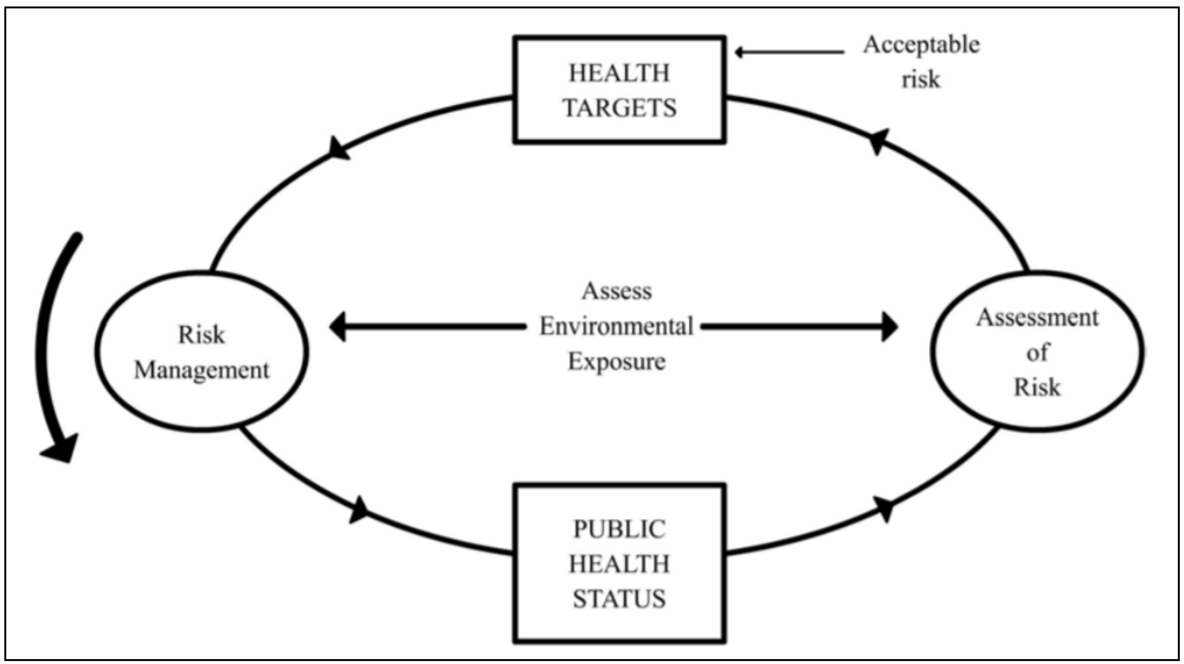
Microbial ecology
Microflora of water

- The autochtonal microflora are presented by microorganisms, which permanently live and are multiplied in water.
- It is more often such species:
- *Micrococcus candicans,*
- *M. roseus,*
- *Sarcina lutea,*
- Bacterium *aquatilis communis,*
- *Pseudomonas fluorescens*.
- The anaerobes represent by species *Clostridium, Bacillus cereus, B. mycoides*.

Microbial Ecology

Regulation of microbiological quality in the water cycle

- Regulation focused on the control of microbiological hazards is important in reducing the incidence of infectious disease.



Howard *et al.*,2001

Microbial Ecology

Regulation of microbiological quality in the water cycle

- Common microbial number of water is examined by inoculation of 1 ml of water in melted and refrigerated up to 50°C in agar in Petri dishes.
- Coli-index is determined by a method of membrane filters or fermentation tests.

Microbial Ecology

Regulation of microbiological quality in the water cycle

- In many countries for a rating of quality of potable water the normative documents or state standards are taken.
- Microbiologic index of safety of potable water are the following:

1. Common microbial number in one ml of water - no more than 100.
2. Number of bacteria of intestinal rod group (coli-index) in 1000 ml of water - no more than 3.
3. Index of fresh fecal contamination, that is quantity(amount) E.coli in 1000 ml of water - absence.
4. Quantity coli-phages (number of colony-forming units) in 1000 ml of water - absence.

- Besides in 25 litters of potable water pathogenic Protozoa (cyst of lamblia, dysenteric amebas, balantidia) and ovum of helmints there should be absent.

Microbial Ecology
Key terms

- Heterotroph: An organism that requires an external supply of energy in the form of food as it cannot synthesize its own.
- Microbiota: A microbiota is "the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space‟.
- Microenvironment (internal): The very small environment in the immediate vicinity of an organism.
- Macroenvironment (external): The large-scale and long-term environment and conditions that affect an organism.

Microbial Ecology
Key terms

Tarantulas

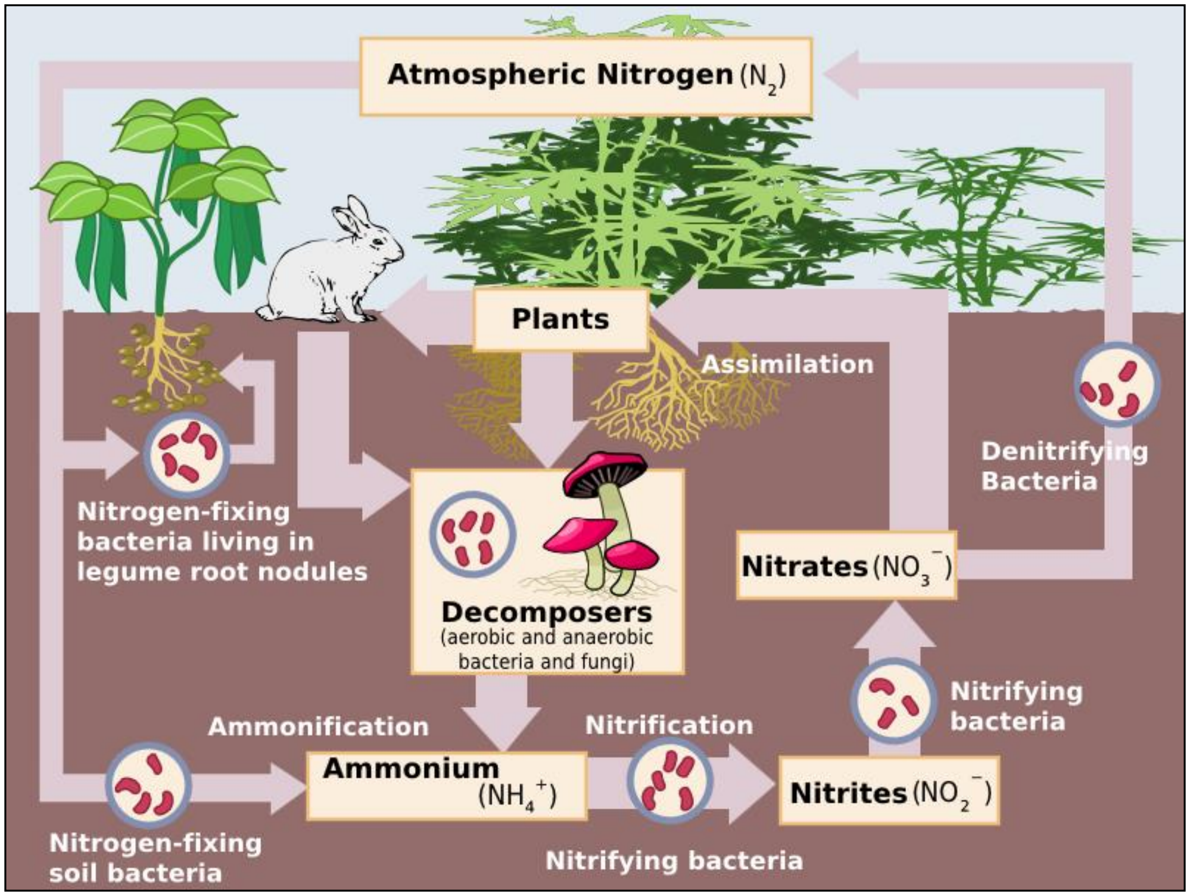
Cacti

- Neutralism: describes the relationship between two species that interact but do not affect each other. It describes interactions where the health of one species has absolutely no effect whatsoever on that of the other. Examples of true neutralism are virtually impossible to prove and most ecologists (as well as textbooks) would agree that this concept does not exist.
- Neutralism the most common type of interspecific interaction. Neither population affects the other. Any interactions that do occur are indirect or incidental. Example: the tarantulas living in a desert and the cacti living in a desert.
- Niche: A function within an ecological system to which an organism is especially suited.

Microbial Ecology

Key terms

Microenvironment vs. Macroenvironment



The Role of Microbes in the Nitrogen Cycle

The processing of nitrogen into a biologically useful form requires the activity of microorganisms.

Microbial Ecology

Key terms

Microbial food web (microbial loop)

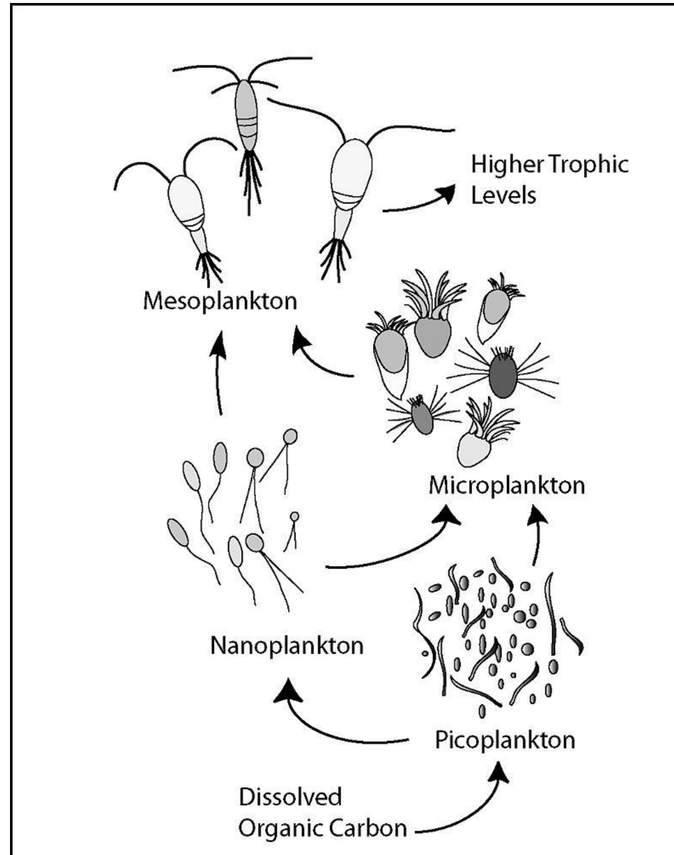
- Food Chain or Food Web
- The sequence of organisms that feed on each other, starting at the phytoplankton and leading to very large organisms.
- Energy is transferred through the biosphere in food chains or food webs.
- The sequence of trophic levels in an ecosystem.

Microbial Ecology

Key terms

Microbial food web (microbial loop)

- Recently the term “microbial food web” has been substituted for the term "microbial loop".
- This means that dissolved organic carbon (DOC) is not available directly to most marine organisms; marine bacteria introduce this organic carbon into the food web, resulting in additional energy becoming available to higher trophic levels.

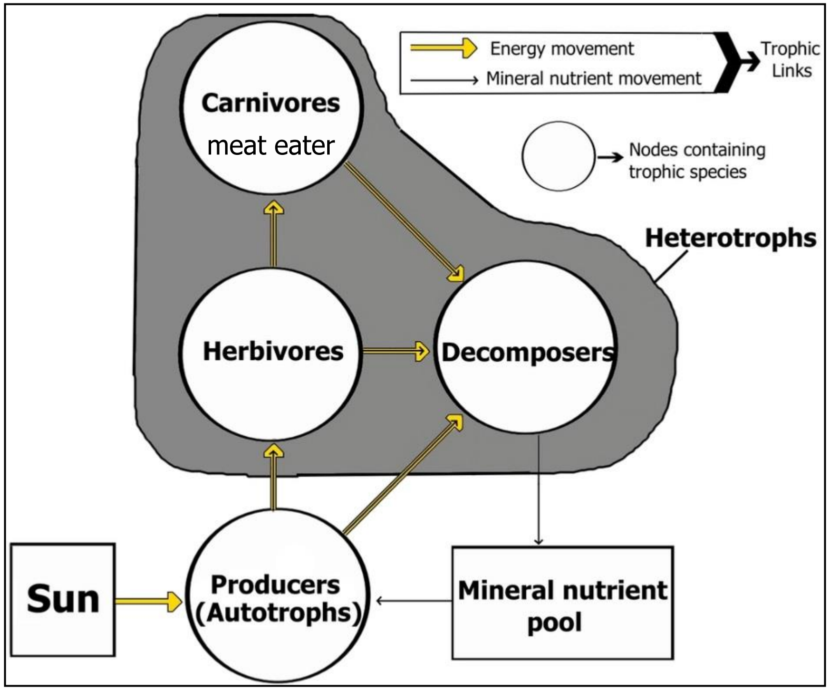


Gaseous, liquid, or solid chemical compounds whose molecules contain carbon. Methane is one of the simplest organic compounds.

Microbial ecology
A simplified food web

- This image shows a simplified food web model of energy and mineral nutrient movement in an ecosystem.

1. Energy flow is unidirectional (noncyclic), and
2. mineral nutrient movement is cyclic.



Boundless.com

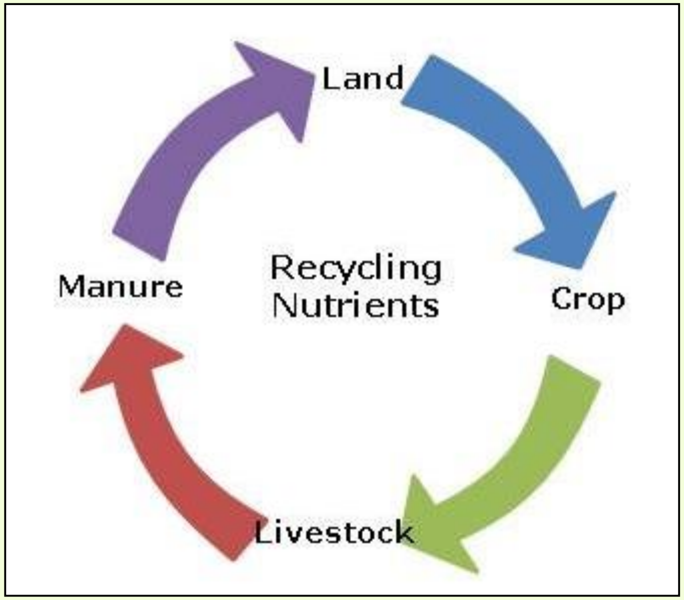
Microbial ecology

Mineral cycles

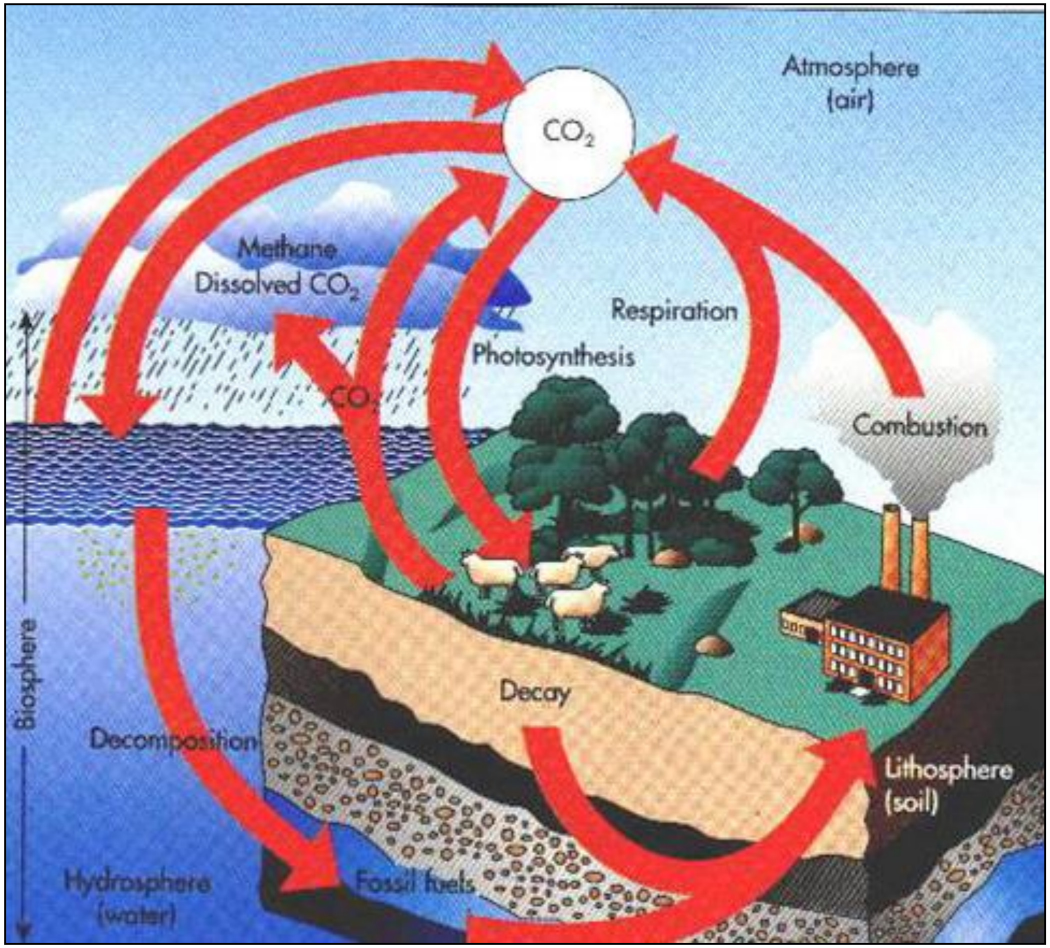
Movement of mineral nutrients is cyclic

- Mineral cycles include:
- carbon cycle,
- sulfur cycle,
- nitrogen cycle,
- water cycle,
- phosphorus cycle,
- oxygen cycle.
- Among others that continually recycle along with other mineral nutrients into productive ecological nutrition.

- The nitrogen cycle, the phosphorus cycle, the sulphur cycle and the carbon cycle all depend on microorganisms in one way or another.



Microbial ecology
Global biogeochemical cycling
Carbon cycle in nature



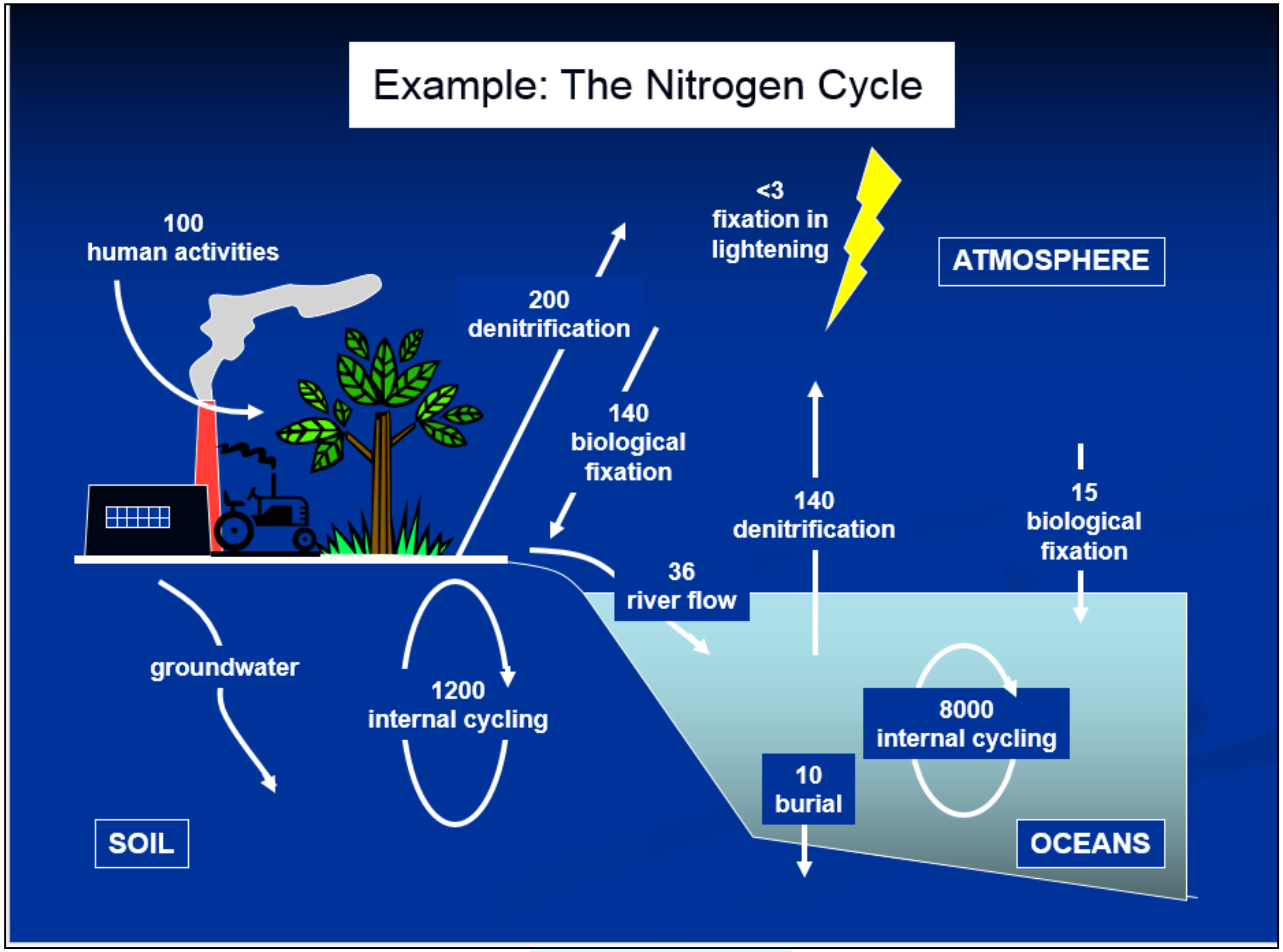
S.I. Klymnyuk

Microbial ecology
Global biogeochemical cycling
The nitrogen cycle

- Nitrogen (N) is one of the building blocks of life: it is essential for all plants and animals to survive.
- For example, the nitrogen gas which makes almost 80% of the earth's atmosphere is unavailable to most organisms, until it is converted to a biologically available form by the microbial process of nitrogen fixation.

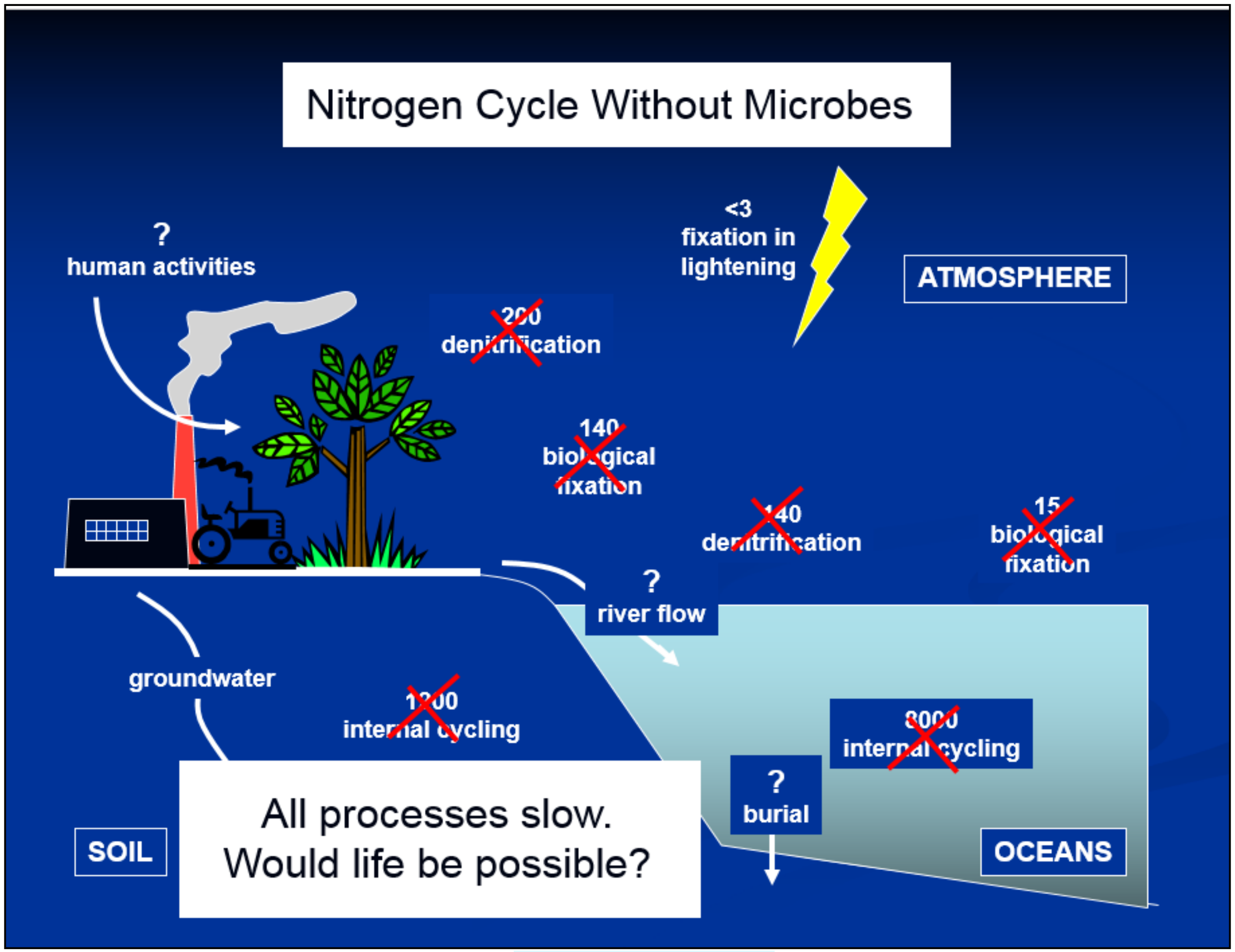
Wikipedia,2016

Microbial ecology
Global biogeochemical cycling
The nitrogen cycle



Martin Polz

Microbial ecology
Global biogeochemical cycling
The nitrogen cycle



Martin Polz

Microbial ecology

Global biogeochemical cycling

The nitrogen cycle

- Humans and most other species on earth require nitrogen in a “fixed,” reactive form. Reactive nitrogen is necessary for the food production process.
- The Haber-Bosch process--an industrial process through which we can fix reactive nitrogen--has allowed food production to keep up with the growing human population, but at a cost to the environment.
- Today, humans create over 2 times as much reactive nitrogen as nature. In contrast, human activity contributes just 5-10% of CO_2 emissions.

Note: Human CO2 emissions upset the natural balance of the carbon cycle.

Microbial ecology
Global biogeochemical cycling
The nitrogen cycle

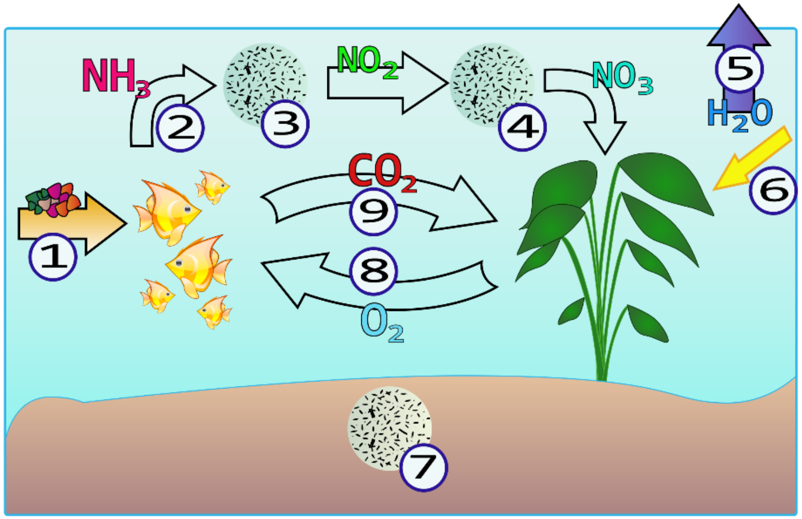
- Much of this reactive nitrogen has accumulated in the environment, where it causes a series of negative impacts to human and ecosystem health.
- Major sources of this reactive nitrogen include agriculture and the burning of fossil fuels.
- This nitrogen pollution causes profound environmental impacts, including smog, acid rain, forest dieback, coastal ‘dead zones’, biodiversity loss, stratospheric ozone depletion and increased greenhouse gases.
- It also affects human health, including respiratory disease and an increased risk for birth defects.

N-Print.org,2011

Microbial ecology
Global biogeochemical cycling
Nitrogen cycle in aquarium

- Legend:

1. Addition of food and nutrients,
2. Production of urea and ammonia by fish,
3. Ammonia is converted to nitrites by beneficial nitrosomonas bacteria,
4. Nitrites are converted to nitrates by beneficial nitrospira bacteria. Less toxic nitrates are removed by plants and periodic water changes.
5. Evaporation.
6. Light.
7. Soil.
8. O_2 produced by plants.
9. CO_2 produced by fish.



Boundless.com

Microbial ecology

Ferric ion (Fe (III) uptake

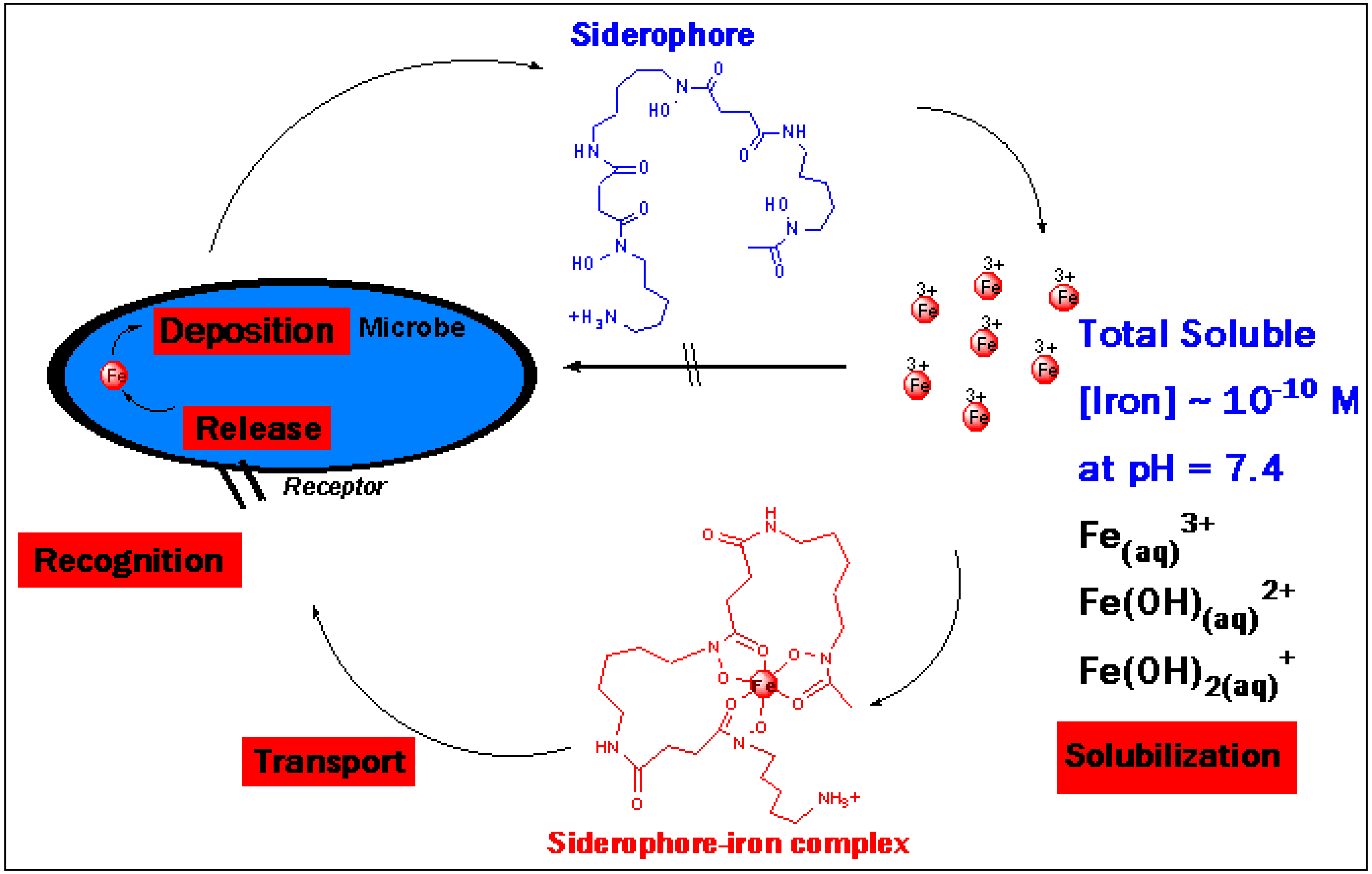
Siderophores

- Many microbes synthesize and excrete low molecular weight ferric iron chelating compounds known as siderophores for the sequestration and uptake of iron.
- Siderophores:

1. Form complexes with ferric iron;
2. Imported the complex into the cell by an ABC (ATP-binding cassette (ABC) transport system.

Siderophores

Iron acquisition by microorganisms



Microbial Ecology

Key terms

- Omnipresence or ubiquity: is the property of being present everywhere.
- Photosynthesis: The process by which plants and other photoautotrophs generate carbohydrates and oxygen from carbon dioxide, water, and light energy in chloroplasts.
- Population: Group of organisms belonging to one species that occur within a defined area.
- Probiotics: are live bacteria and yeasts that are good for your health, especially your digestive system. We usually think of bacteria as something that causes diseases. But your body is full of bacteria, both good and bad. Probiotics are often called "friendly", "good" or "helpful" bacteria because they help keep your gut healthy (Russo,2017).

Microbial Ecology

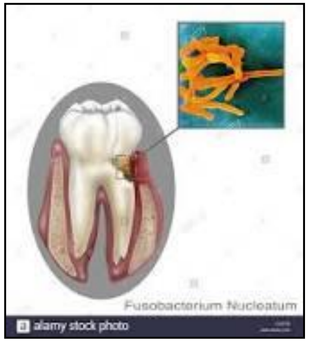
Key terms

- Protista: composed of "organisms which are unicellular or unicellular-colonial and which form no tissues. Protists are the members of an informal grouping of diverse eukaryotic organisms that are not animals, plants or fungi.
- Species: Groups of interbreeding natural populations that are reproductively isolated from other such groups.
- Succession: Continuous replacement in time of one community by another community. These changes in communities are controlled by both the dynamic nature of the abiotic and biotic components of the ecosystem.
- Ecological succession is the process of change in the species structure of an ecological community over time. The time scale can be decades (for example, after a wildfire), or even millions of years after a mass extinction.

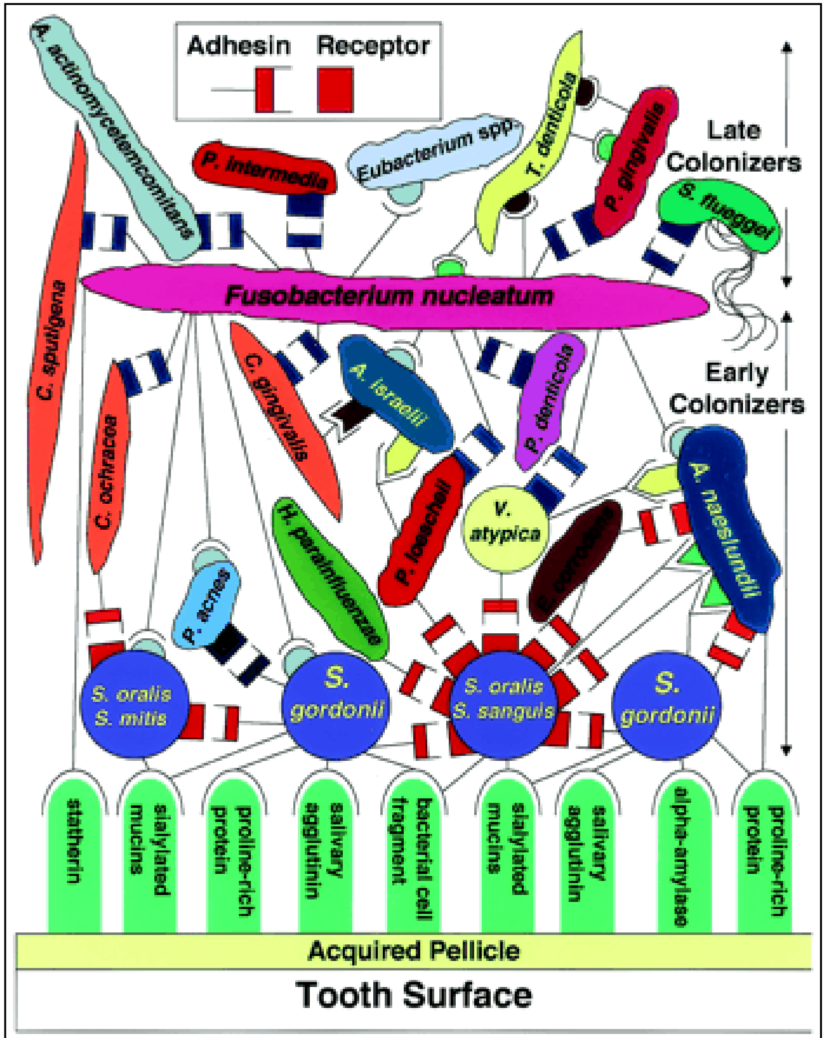
Ecological succession

Tooth surface

In vitro biofilm



- 3° colonizers (Gram-)
- *Porphyromonas gingivalis*
- 2° colonizers (Gram-)
- Bridge species – *Fusobacterium nucleatum*
- Bind other bacteria.
- 1° colonizers (Gram+)
- Streptococci bind pellicle proteins from saliva DENT 5302



Kolenbrander *et al.*,2002

Microbial Ecology

Key terms

- Succession:

1. Autogenic succession:
   In ecology, it is succession driven by the biotic components (plants, animals,…). Biotic factors also include human influence, pathogens and disease outbreaks. Each biotic factor needs energy to do work and food for proper growth) of an ecosystem.
2. In contrast, allogenic succession is driven by the abiotic components (temperature, light intensity, moisture and water levels, air currents, carbon dioxide levels and the pH of water and soil) of the ecosystem.

Microbial Ecology

Key terms

- Transmission:
- Microorganisms are spread in nature by active and passive dispersal.

1. Active transmission: In fungi by growth of hyphae, which is limited to short distance. It is also possible by motility. This applies flagellated bacteria, to zoospores of fungi, to flagellated and ciliated protozoa, and to gliding microorganisms.
2. Passive transmission: Occurs through air, water, and by means of vectors.

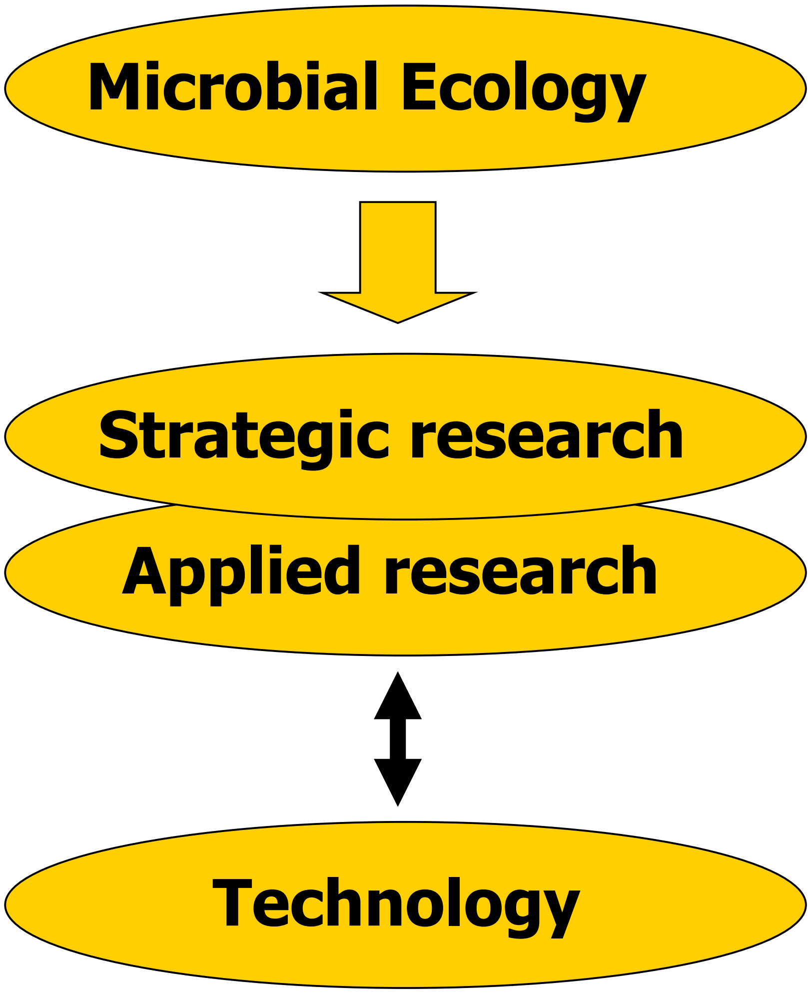
Microbial Ecology
Key terms

- Urban: Human settlements are classified as rural or urban depending on the density of human-created structures and resident people in a particular area.
- An urban area: is a human settlement with high population density and infrastructure of built environment.
- Ubiquitous: Being everywhere at once: omnipresent.

Laboratory of Microbial Ecology and Technology (LabMET)

Research topics and expertise

Mission



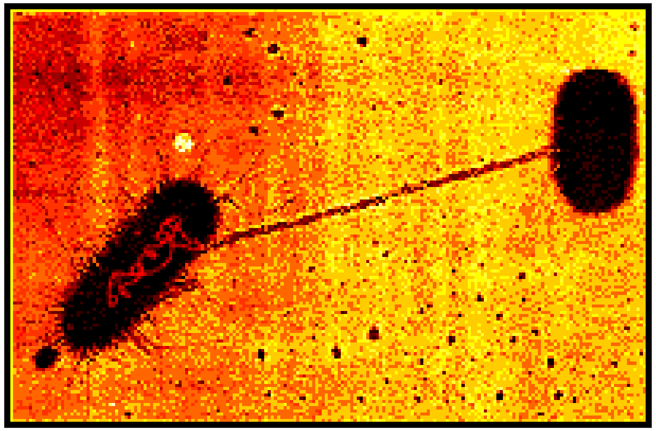
Laboratory of Microbial Ecology and Technology (LabMET)
Research topics and expertise
General overview

1. Strategic research
2. Applied research
3. Tools and instruments

Willy Verstraete

Laboratory of Microbial Ecology and Technology (LabMET)
Research topics and expertise
Strategic Research

- Microbial communities
- Quorum sensing
- Electron shuttling
- Horizontal gene transfer
- Metabolomics



Applied Research

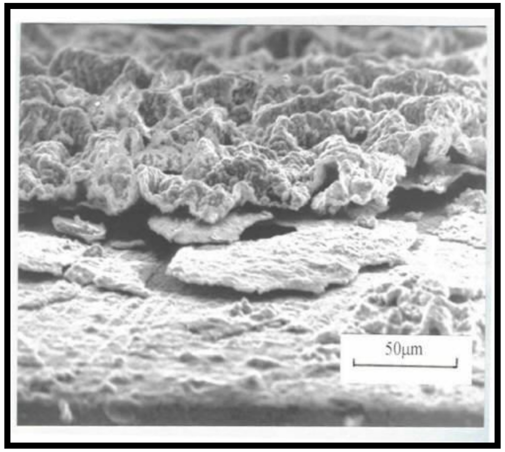
- Environmental Microbiology
  - Wastewater
    - Microbial fuel cells
    - Biodegradation
    - Anaerobic treatment
    - Nitrogen removal strategies
    - Minimizing wast sludge

Applied Research

- Environmental Microbiology
  - Soil / Sediments
    - Bioprecipitation of catalytic particles
    - Anaerobic removal of organochlorine contaminants
    - Soil and river sludge clean-up
    - Pesticide degradation and ecotoxicology

Applied Research

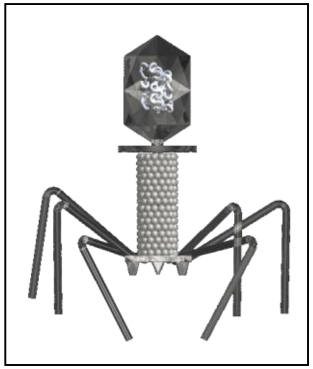
- Environmental Microbiology
  - Solid Wastes
    - Solid waste treatment
    - De-icing
  - Buildings and structures
    - Biologically mediated $CaCO_3$ formation
    - Microbial induced corrosion
  - Air
    - Indoor air pollution
    - Biotrickling filtration



Willy Verstraete

Applied Research

- Gastro-intestinal Microbiology
  - Functional foods
    - Pro- / pre- / synbiotics
    - Bioactivation of food components
    - Rumen microbiology
  - Risk assessment
    - Environmental contaminants
    - Toxic food processing metabolites
  - Phage therapy



Synbiotics refer to food ingredients or dietary supplements combining probiotics and prebiotics in a form of synergism, hence synbiotics. Probiotics are microorganisms that are believed to provide health benefits when consumed. In diet, prebiotics are typically non-digestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate he growth or activity of advantageous bacteria such as lactic acid bacteria.

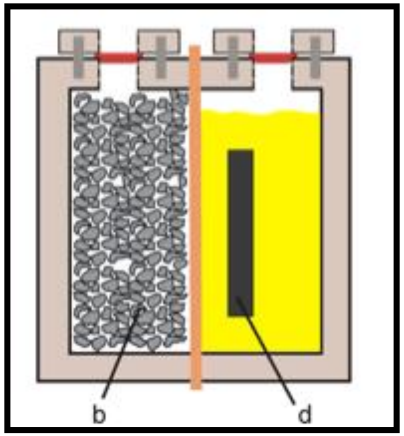
Applied Research

- Foodchain Microbiology
  - Drinking water
    - Hygienisation
    - Water recycling
    - Pathogen abatement
  - Aquaculture systems
- Habitat research
  - Epiphytes on grain
  - Space station life cycles
  - Deep sea methane oxidation

Tools and instruments

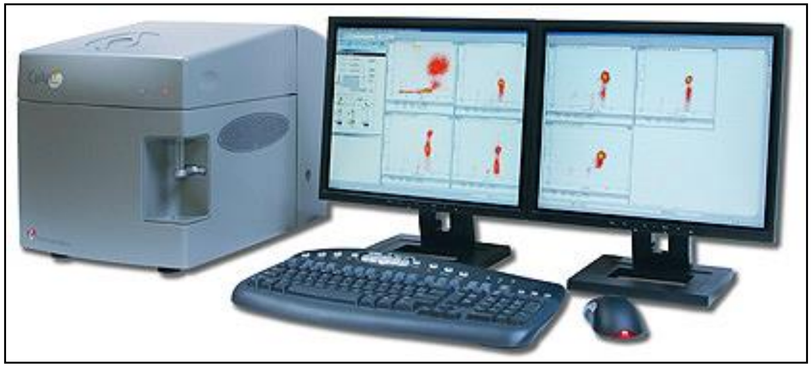
Reactor Technology

- Simulator of the Human Intestinal Microbial Ecosystem (SHIME)
- Activated sludge systems
- Upflow Anaerobic Sludge Bed reactors
- Microbial Fuel cells
- Membrane reactors
- Rotating disc reactors
- Dialysis reactors



Tools and instruments
Molecular Analysis

- PCR
- DGGE
- FISH
- Realtime PCR
- Cloning
- Flow Cytometry.



Tools and instruments
Microbial analysis

- Epifluorescence and light microscopy
- Growth kinetics
- Microbial isolations and enrichments
- Metabolic activity
- Bioassays
- Biodegradation assays.

Tools and instruments
Physico-chemical analysis

- Gas chromatography
- HPLC
- Ion chromatography
- Spectrophotometry
- Atom absorption
- BOD, COD, TSS, VSS, NOX, TOC,…

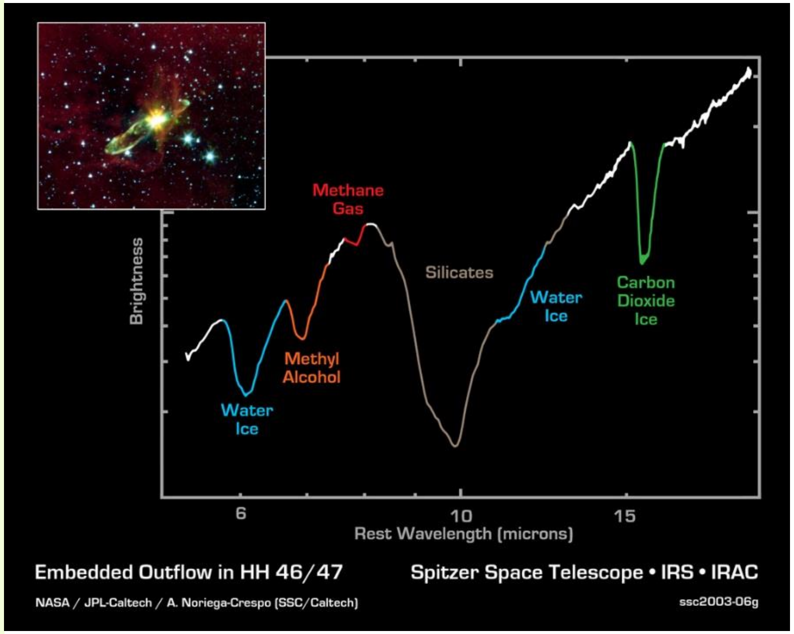
Tools and instruments
Organic molecules detected in space

- NASA's Spitzer Space Telescope has lifted the cosmic veil to see an otherwise hidden newborn star, while detecting the presence of water and carbon dioxide ices, as well as organic molecules.
- The Spitzer image (inset) was obtained with the infrared array camera.

Tools and instruments

Organic molecules detected in space

- The primary image shows a spectrum obtained with Spitzer's infrared spectrograph instrument, stretching from wavelengths of 5.5 microns to 20 microns.
- Spectra are graphical representations of a celestial object's unique blend of light.
- Characteristic patterns, or fingerprints, within the spectra allow astronomers to identify the object's chemical composition.



Boundless.com

Microbial ecology

Human microbiome

- The innumerable microbes living in and on our bodies are known to affect human wellbeing, but our knowledge of their role is still at the very early stages of understanding.
- Human Microbiome is a new open access journal dedicated to research on the impact of the microbiome on human health and disease.
- Topics covered will include: the repertoire of human-associated microbes, therapeutic intervention, pathophysiology, experimental models, physiological, geographical, and pathological changes, and technical reports; genomic, metabolomic, transcriptomic, and culturomic approaches.

Microbiomes

Human Microbiome Project (HMP)

- The Human Microbiome Project (HMP) was a United States National Institutes of Health (NIH) initiative with the goal of identifying and characterizing the microorganisms which are found in association with both healthy and diseased humans (the human microbiome).

Logo of the Human Microbiome Project.

Wikipedia,2016

Human microbiomes

Human Microbiome Project (HMP)

- Projects:
- The human microbiome Project:

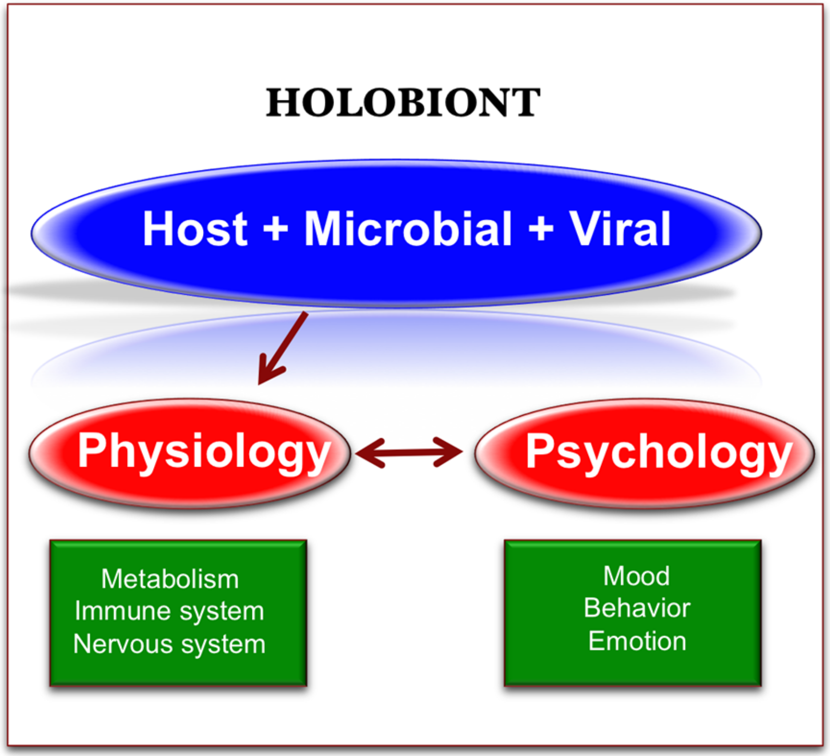
1. The human microbiome: at the interface of health and disease.
2. The skin microbiome: potential for novel diagnostic and therapeutic approaches to some diseases.

Human holobiome

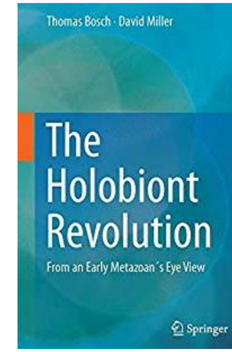
The host genome and associated microbiome

What's a holobiont?

- All animals and plants have developed symbiotic relationships with microorganisms.
- Holobiont is the term used to refer to the host plus all its associated microorganisms.
- Anatomically only approximately 50% of cells and 1% of genes in the human holobiont belong to the host organism.



Human microbiome vs. Human holobiome



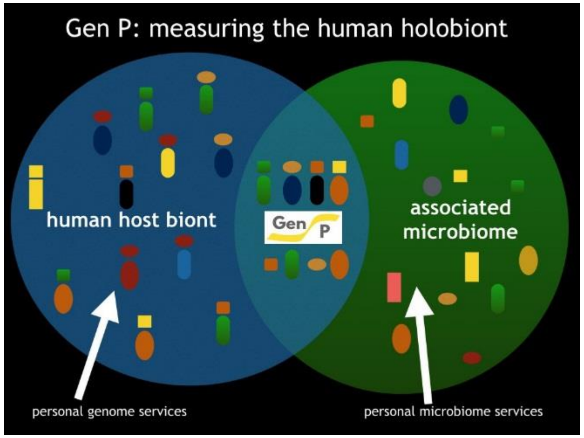
- The microbiome is the tens of thousands of bacteria that make up our microbiotic flora. We have a microbiome in our mouth, gut, yoni region, and on our skin. They have everything to do with our immunity, how we digest our meals, the quality of our skin, our mood, our odor, our energy, what foods we crave. The list goes on and on.
- But our holobiome (the eukaryote organism with its microbiome) is this group of microbes in our gut plus the ones in our skin as well as the cloud of bacteria that walks through life with us.

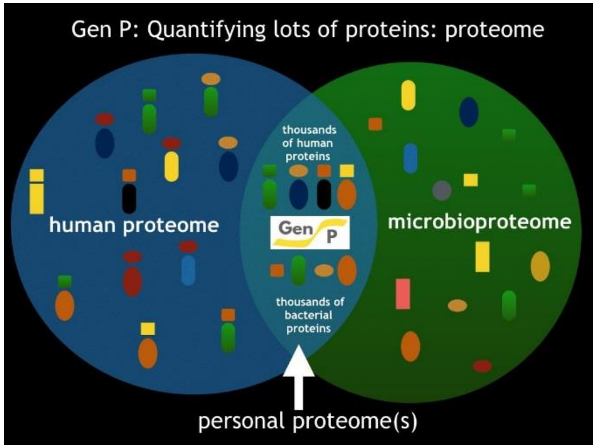
Living,2018;..

Human holobiome

The host genome and associated microbiome

- So of course, just as we are in constant communication and the ride of life with our precious bacteria, we will be affected by their health and theirs by ours.
- If you keep your holobiome happy, you will probably meet wonderful people (each little bacteria holds a certain energy depending on its health), have more energy, enjoy brighter skin, and have an overall sense of well-being imparted on you





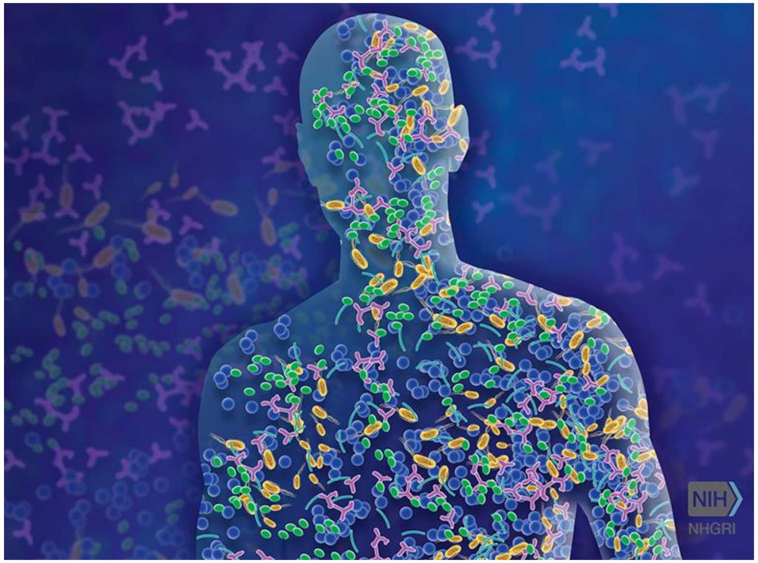
Living,2018;Atilla,2017

Human holobiome

The host genome and associated microbiome

Eukaryote evolution

- There has been growing awareness of the importance of studying the holobiome – the eukaryote organism with its microbiome – for the understanding of eukaryote evolution.



The human holobiont.
Holobionts are defined as the host plus of all its microbial symbionts, including transient and stable members.

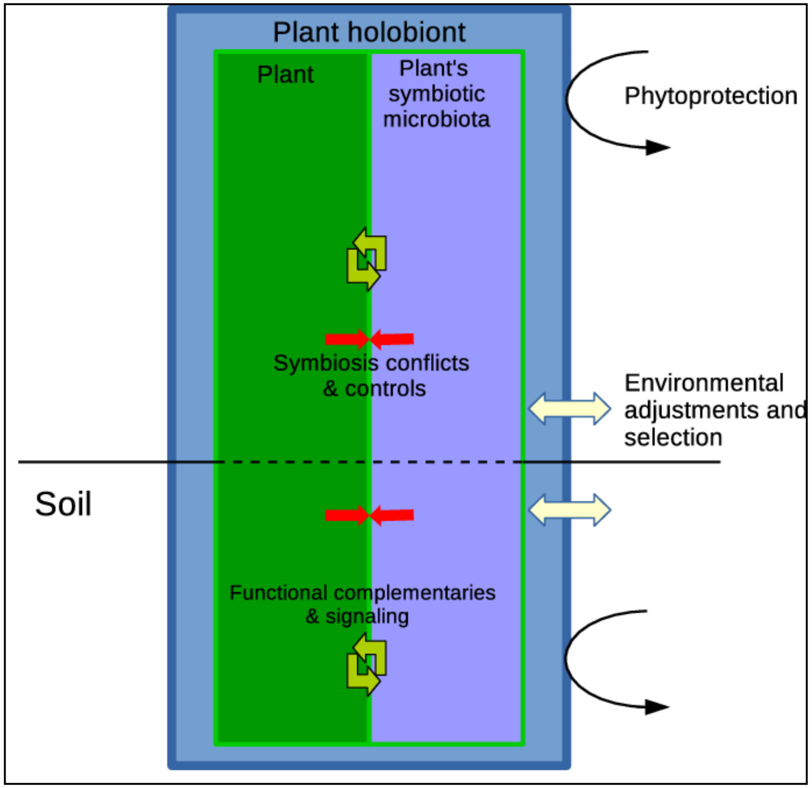
Feldman and Kolodny,2017;...

Plant holobiome

The host genome and associated microbiome

- Scheme of the plant holobiont and related key interaction aspects both in term of:

1. Evolution, and
2. functioning.



Vandenkoornhuyse *et al.*,2015

Plant holobiome

The host genome and associated microbiome

The Populus holobiont

- Microorganisms serve important functions within numerous eukaryotic host organisms.
- An understanding of the variation in the plant niche-level microbiome, from rhizosphere soils to plant canopies, is imperative to gain a better understanding of how both the structural and functional processes of microbiomes impact the health of the overall plant holobiome.
- Using *Populus* trees as a model ecosystem, we characterized the archaeal/bacterial and fungal microbiome across 30 different tissue-level niches within *Populus* individuals using 16S and ITS2 rRNA gene analyses.

Cregger *et al.*,2018

Human microbiota

Normal microbiota on and in the human body

- Microorganisms begin colonization in and on the surface of the body soon after birth.
- **Normal microbiota:** microorganisms that establish permanent colonies inside or on the body without producing disease.
  - *Staphylococcus* on epidermis and mucus membranes;
  - *Escherichia coli* in colon.
- **Transient microbiota:** are microbes that are present for various periods and then disappear.
  - Although these types of organisms may colonize a host and reproduce briefly, the defenses of the human body do not allow them to become permanent.

As nouns the difference between microbiota and microbiome is that microbiota is the microbial flora harbored by normal, healthy individuals while microbiome is the genetic information (genomes) of a microbiota.

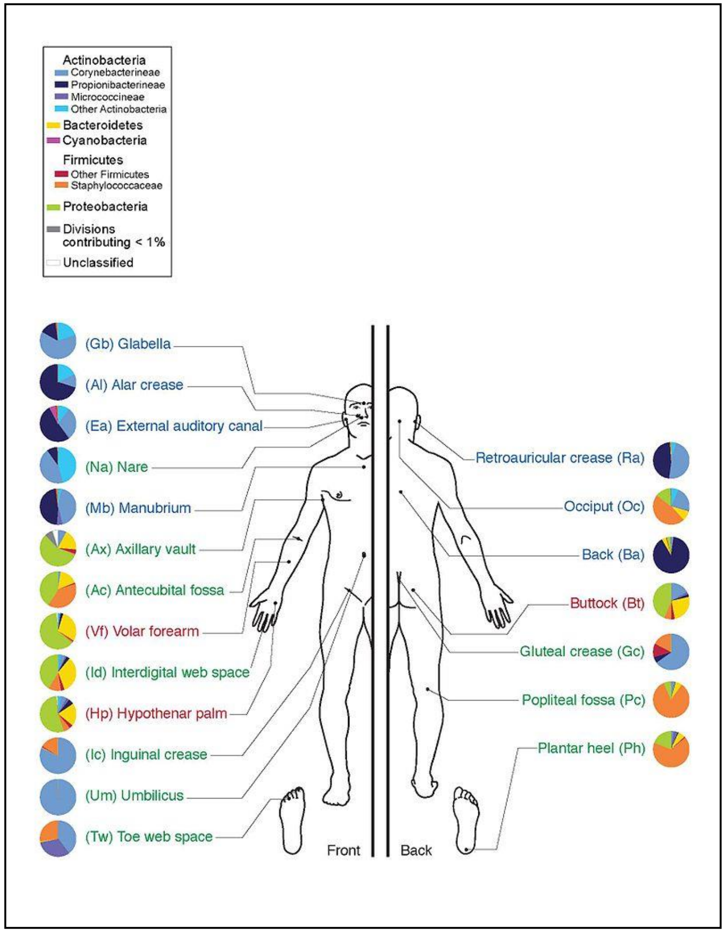
Human microbiota
Microbiome and human body environment
Ecosystems

- Microbial community vary at different body sites on the same person (different ecosystem).
- Bacteria of a specific body site from different persons have more common characteristic (common ecosystem).
- The microbiome of a healthy and diseased person looks very different (rearranged ecosystem).

Human microbiomes

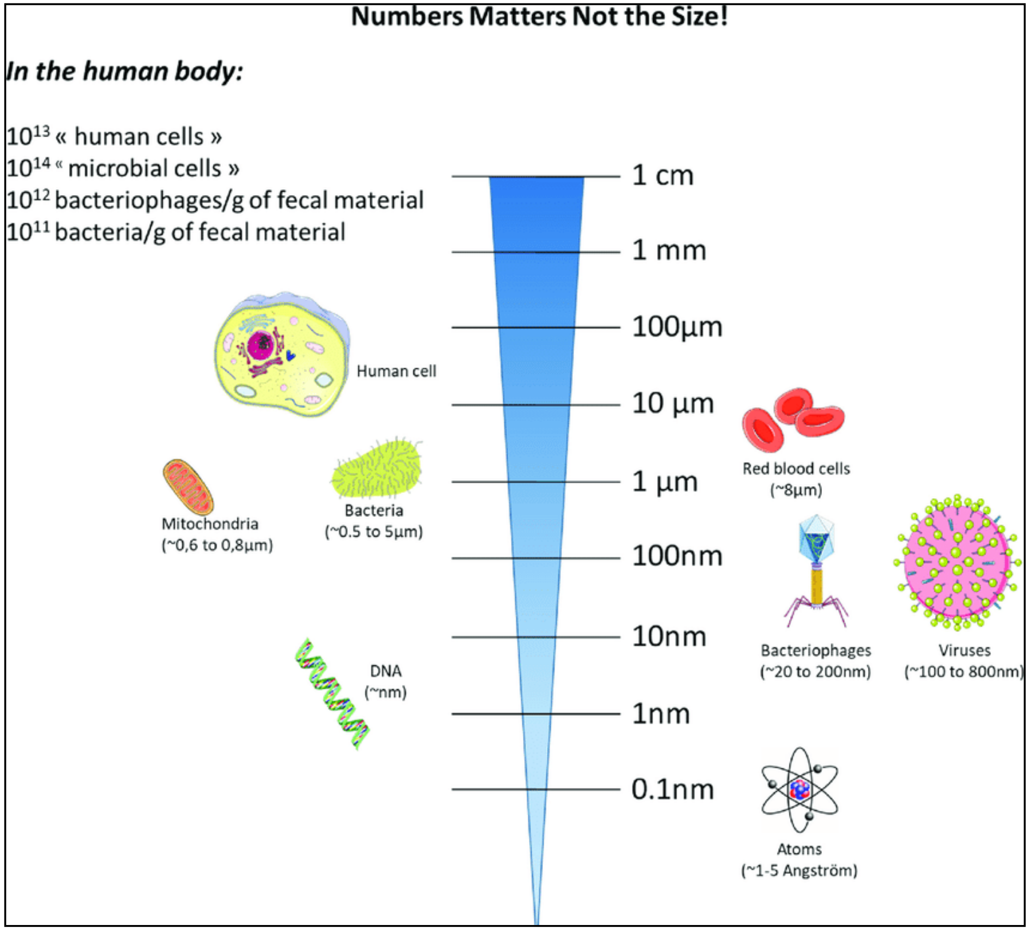
Humans carry more bacterial cells than human ones

- You are more bacteria than you are you, according to the latest body census.
- All the bacteria living inside you would fill a half-gallon jug; there are 10 times more bacterial cells in your body than human cells.
- In a new study it was estimated the average human male is made of 30 trillion cells and contains about 40 trillion bacteria, most of which reside in his digestive tract.



Wenner,2007; Wikipedia,2016;Greshko,2016

Relative sizes of major host cells and their components versus those of bacteria and viruses



Cani, 2018

Human microbiomes

Humans carry more bacterial cells than human ones

- The infestation begins at birth: Babies ingest mouthfuls of bacteria during birthing and pick up plenty more from their mother's skin and milk-during breast-feeding, the mammary glands become colonized with bacteria.
- "Our interaction with our mother is the biggest burst of microbes that we get.
- That's just for starters: Throughout our lives, we consume bacteria in our food and water "and who knows where else“.

Wenner,2007

Human microbiomes

Humans carry more bacterial cells than human ones

- Starting in the mouth, nose or other orifices, these microbes travel through the esophagus, stomach and/or intestines—locations where most of them set up camp.
- Although there are estimated to be more than 500 species living at any one time in an adult intestine, the majority belong to two phyla:

1. The Firmicutes (which include *Streptococcus*, *Clostridium* and *Staphylococcus*), and
2. The phylum Bacteroidetes (which include *Flavobacterium*).

Wenner,2007

Human microbiomes

Humans carry more bacterial cells than human ones

- The bacterial body has made another contribution to our humanity- genes.
- Soon after the Human Genome Project published its preliminary results in 2001, a group of scientists announced that a handful of human genes—the consensus today is around 40-appear to be bacterial in origin.
- The question that remains, however, is how exactly they got there.

Wenner,2007

Human microbiomes

Humans carry more bacterial cells than human ones

- Some scientists argue that the genes must have been transferred to humans from bacteria fairly recently in evolutionary history, because the genes aren't found in our closest animal ancestors.
- Others argue that they may be ancient relics from evolutionary events that took place early in our species's history and, for reasons unknown, the genes were lost in these ancestors.
- It's impossible to know for sure at this point.

Wenner,2007

Human microbiomes

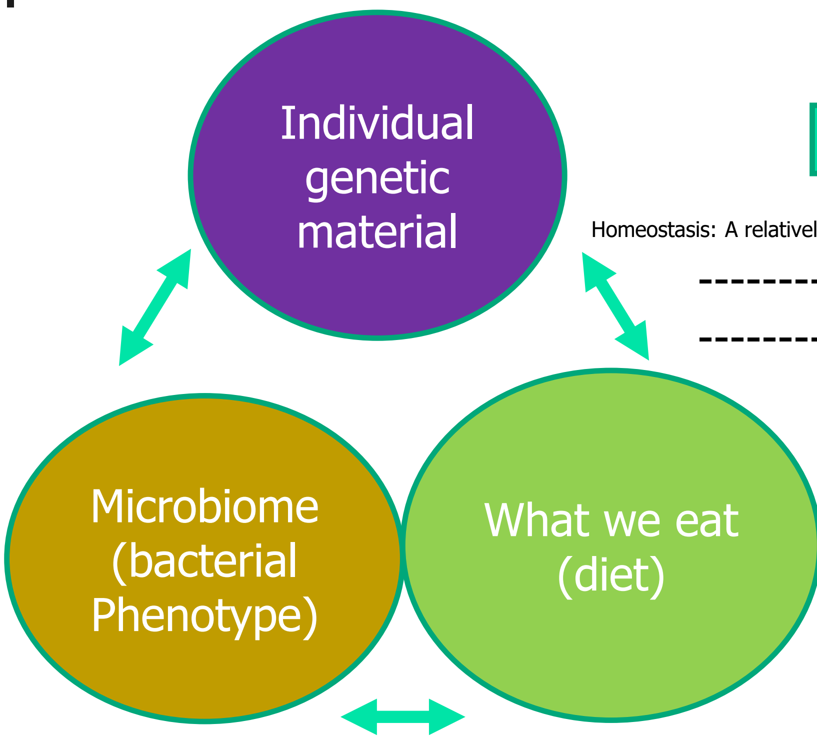
Humans carry more bacterial cells than human ones

- One thing is for sure: our lives and even our identities are more closely linked to the microbial world than we may think.
- Bacteria do a lot to keep us healthy, and scientists are just beginning to uncover their valuable secrets.
- As U.I.'s Bohach says: "We do not completely understand the full impact of our bacterial flora on our health and physiology."

Wenner,2007

Microbiome and metabolic activity

What is a healthy Microbiome?



Health

Homeostasis

Low risk of allergies
Infection resistance

Homeostasis: A relatively stable equilibrium between interdependent elements.

Diseases

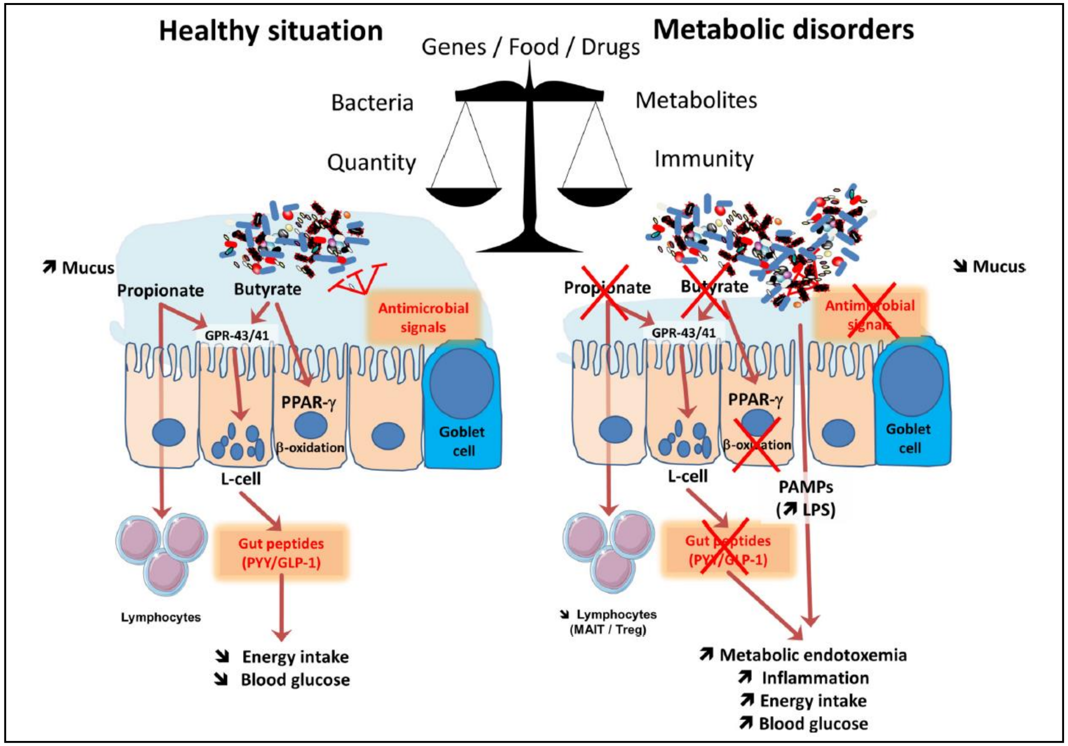
Perturbation

Allergies
Metabolic syndrome
Obesity
Infections

One size does **NOT** fit all - Each person's microbiome is unique.

Major mechanisms involved in the crosstalk between microbes and host: impact of metabolism

- The balance between healthy and pathological situations (e.g., metabolic disorders) is crucial. This is under the tight influence of several factors including the genes, food and drugs.



Cani, 2018

Major mechanisms involved in the crosstalk between microbes and host: impact of metabolism

- This left part of the figure shows that in healthy situation, the composition of the gut microbiome is associated with a higher mucus layer thickness, the production of antimicrobial signals and different short-chain fatty acids such as butyrate and propionate. Both butyrate and propionate bind to G protein coupled receptors (GPR)-43 and GPR-41 expressed on the enteroendocrine L-cells thereby stimulating the secretion of gut peptides such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). This effect contributes to reduce food intake and to improve glucose metabolism.
- Propionate can also bind to GPR-43 expressed on lymphocytes in order to maintain appropriate immune defence. Butyrate activates peroxisome proliferator-activated receptor-γ (PPAR-γ) leading to beta-oxidation and oxygen consumption, a phenomenon contributing to maintain anaerobic condition in the gut lumen.

Cani, 2018

Major mechanisms involved in the crosstalk between microbes and host: impact of metabolism

- As depicted on the right part of the figure, during metabolic disorders, changes in the gut microbiome are linked with a lower mucus thickness, decreased antimicrobial defense and butyrate and propionate production. As a consequence, L-cells secrete less gut peptides.
- The lack of PPAR-γ activation lead to higher oxygen available for the microbiota at the proximity of the mucosa and increases the proliferation of Enterobacteriaceae. The decrease in propionate also contribute to the lower abundance of specific T cells (mucosal-associated invariant T cells (MAIT) and Treg) in the lamina propria of the gut. Altogether, such changes in the microbial environment and metabolites induce a leakage of pathogen associated molecular patterns (PAMPs) such as the lipopolysaccharide (LPS) that are increased in the blood, and trigger low-grade inflammation.

Cani, 2018

Human microbiomes

Relationships between the normal microbiota and other microorganisms

- **Microbial antagonism:**

1. Normal microbiota can prevent pathogens from causing an infection by competing for nutrition.
2. Produce substances harmful for invading organisms.

- E.g. *E. coli* produce bacteriocines.

1. Affecting condition as pH or available oxygen.

- **Cooperation among microorganisms:**
- In some situations, one microorganism makes it possible for another to cause a disease or produce more severe symptoms.
- e.g. Streptococci that colonize the teeth.

Human microbiomes
How to take care of our “second genome”

- **We are what we eat:**
- **Probiotics** – application of life microbial culture.

- Typically lactic acid producing bacteria ingested to aid digestion and protect intestine from pathogens.
- **Prebiotics** - refer to chemicals that induce the growth and/or activity of commensal microorganisms.

- Mix of bacteria instead of antibiotics

- **Fecal microbiota transplantation (**FMT**):**
- Bacteriotherapy for restoration of normal microbiota.

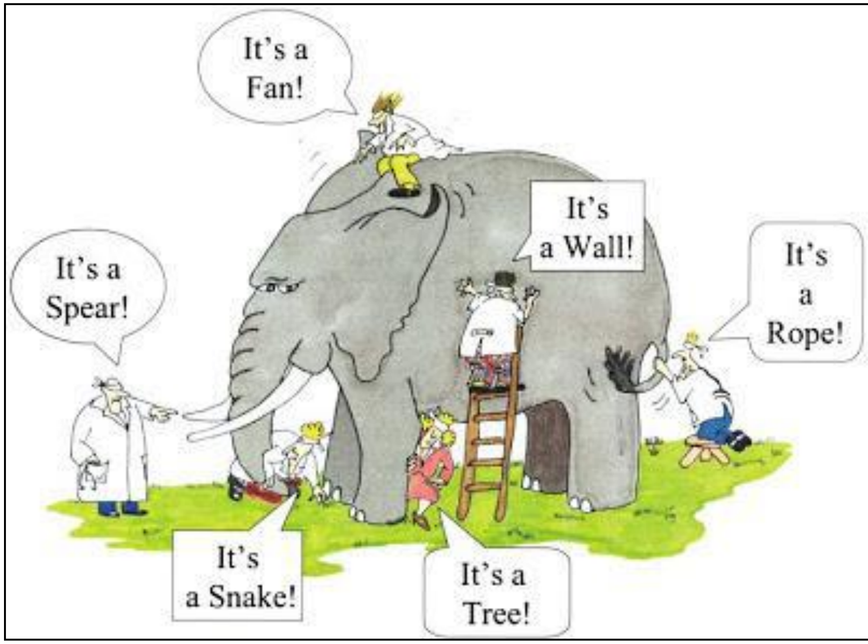
Phytobiomes

What is the phytobiome?

Plant microbiomes are components of phytobiome

- Understanding and application of microbiomes to advance agriculture requires:

1. Interdisciplinary, systems level approaches;
2. Consideration of interactions in context (the phytobiome).



We have to remember that what we observe is not nature in itself, but nature exposed to our method of questioning. - Werner Heisenberg (Theoretical physicist).

Phytobiomes (probiotic)

Microbiomes

Plant microbiomes are components of phytobiome

- The plant microbiome ("Phytobiome") is a relatively new field of study with intriguing potential applications that are in the early stages of development.
- The phytobiome is analogous to probiotic (beneficial microbes) studies in humans (e.g. gut microbiome).
- The plant microbiome is an assemblage of microbes living in, on and around plants.
- These biomes function as a community of microoganisms with predictable compositions and are partners for life.

Dickman,2015

Phytobiomes

Microbiomes

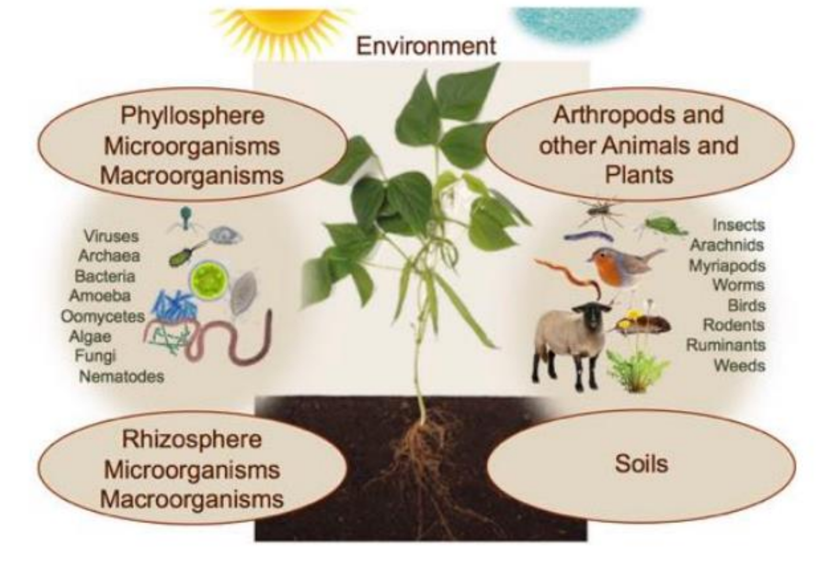
Plant microbiomes are components of phytobiome

Berendsen *et al.*,2012

Phytobiomes website

A Roadmap for phytobiomes research and translation

- PHYTOBIOMES consist of plants, their environment, and their associated micro- and macroorganisms. These organisms, which may be inside, on the surface, or adjacent to plants, include a wide diversity of microbes (viruses, bacteria, fungi, oomycetes, and algae), animals (arthropods, worms, nematodes, and rodents), and other plants.



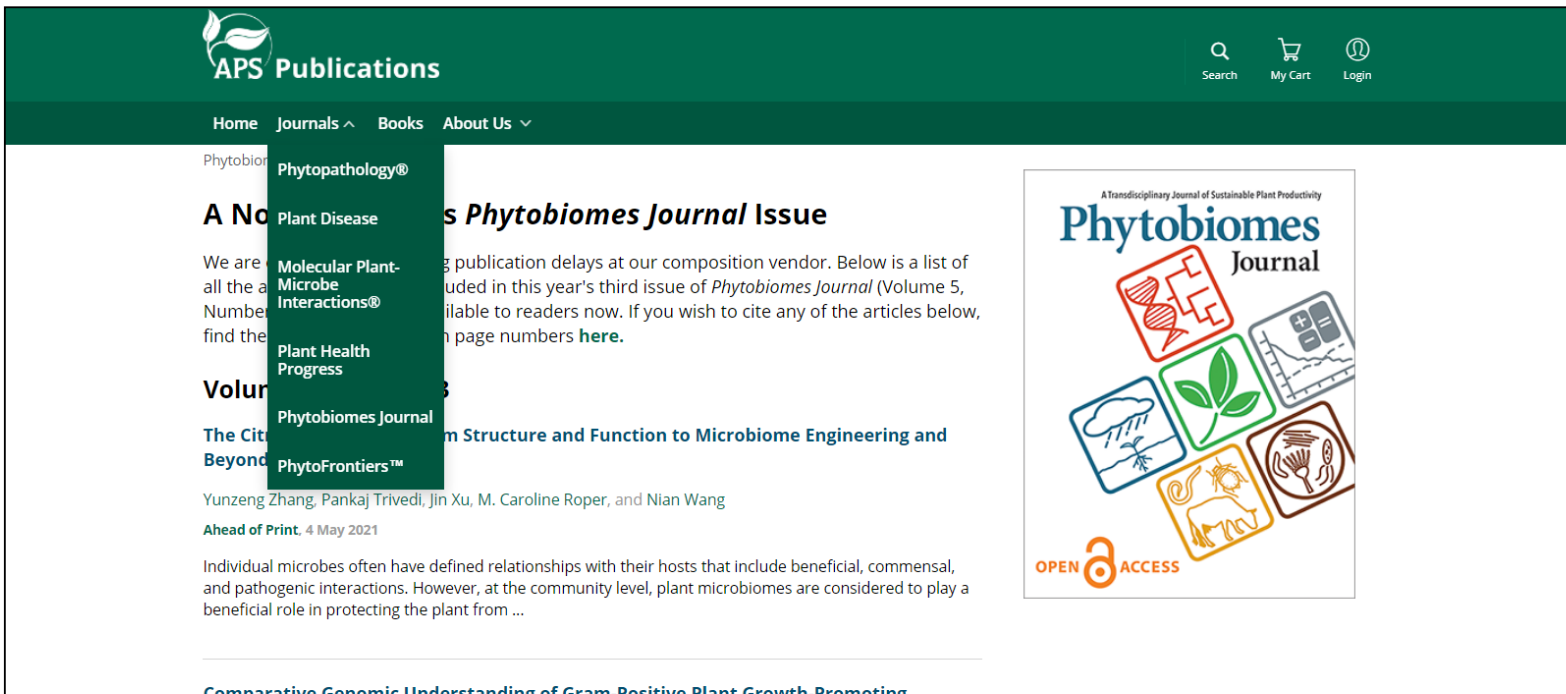
Phytobiomes website: www.phytobiomes.org

The environment includes the physical and chemical environment influencing plants and their associated organisms, and therefore, the soil, air, water, and climate. The sphere of relevance of phytobiomes is quite broad, spanning from crops (commodity crops, fruits, vegetables, forest, and specialty and bioenergy crops), rangelands, grasslands, and natural ecosystems to consumer products, including the quality, nutritional value, and safety of our foods.

Phytobiomes Journal

The American Phytopathological Society (APS)

- Phytobiomes is a new open-access journal published by APS.
- This high-quality journal focuses on transdisciplinary research that impacts the entire plant ecosystem

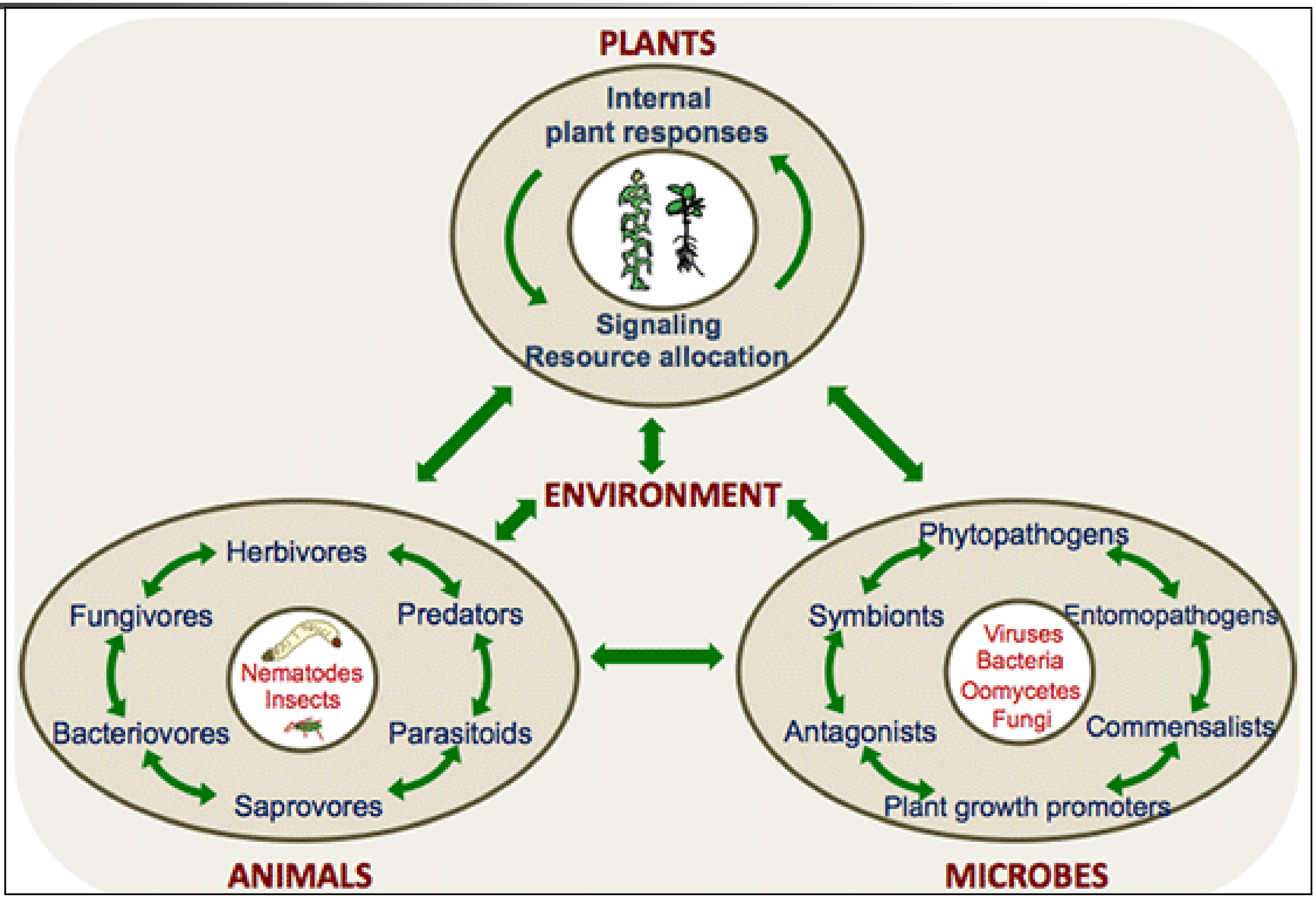


Phytobiomes
Microbiomes
Plant microbiomes are components of phytobiome

- Phytobiomes consist of:
1. plants,
2. their environment, and
3. their associated communities of organisms.
- Interactions within phytobiomes have profound effects on soil, plant and agroecosystem health.
- Knowledge of the phytobiomes network of can be translated into new tools for agroecosystem management.

Phytobiome

Dynamic and multitrophic interactions occur among the organisms and between organisms and the environment in phytobiomes



Berendsen *et al.*,2012

Phytobiomes

Microbiomes

Plant microbiomes are components of phytobiome

- The entire system of factors that affect or are affected by plants, including:

1. Microbial communities (microbiomes) of the plants, soils, and animals;
2. Insects, nematodes, and other animals;
3. Plants;
4. Soils;
5. Environment.

- This entire system influences the health and productivity of plants, plant ecosystems, and all who consume the plants and plant products.

Berendsen *et al.*,2012

Phytobiomes
Microbiomes
Role of microbes in soil fertility and human health

- Human (and animal) health depends to a large extent on soil related microbial populations in the gut. Soil fertility depends on healthy microbial populations in the soil.
- The microbes in the human and animal gut and the soil microbes are related because there is a constant exchange via food ingestion.
- This is known as the soil food web.

Phytobiomes

Microbiomes

Role of microbes in soil fertility and human health

- Research has shown that **soil microbial life is becoming less diverse all over the world because of use of chemical fertilzers, herbicides, insecticides and other modern agricultural practices, leading to low quality crops and low quality food**.
- It also becomes more and more clear that the microbial flora in the gut is directly linked to immune competence and there are indications that unhealthy gut flora is linked to human illnesses like Crohn's disease, some forms of cancer, Alzheimer and depression, to name a few.
- The purpose of this collection is to aggregate key papers on the topics discussed above, with the aim of raising awareness on the importance of soil-microbial-human interactions.

Olijhoek *et al.*,2015

Phytobiomes

Microbiomes

Role of phytobiomes in plant disease control

- Plant disease may be influenced by phytobiome members beyond the host and the pathogen.
- Host defenses may be modulated by microbes and insects.
- We will focus on recent discoveries of the:

1. influence of plant-associated insects and microbes on plant disease outcomes, and on
2. how this knowledge may be translated into applications for disease management.

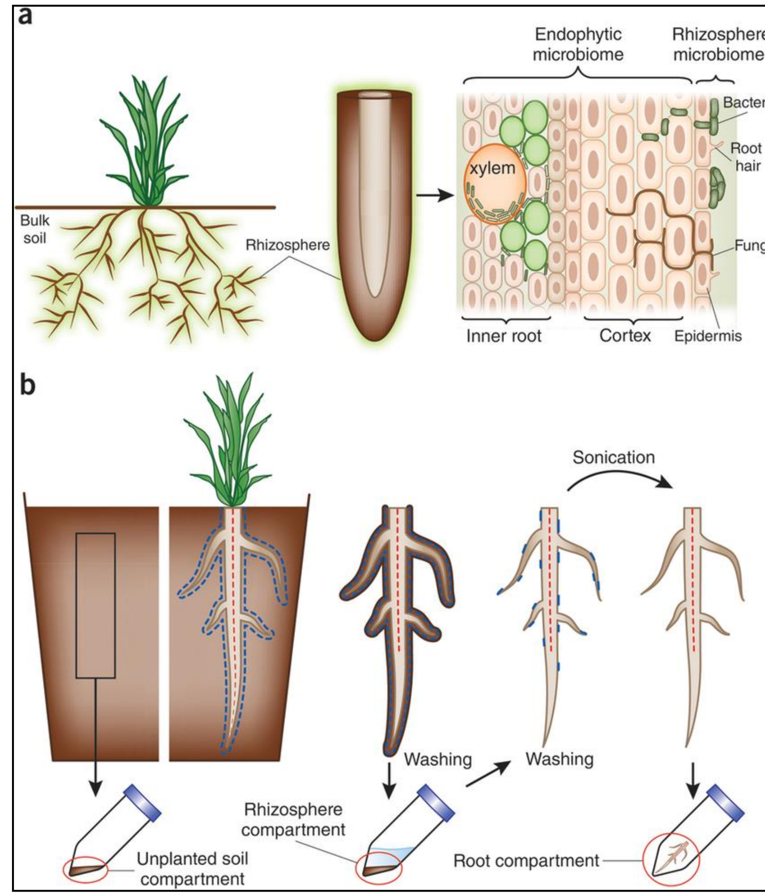
Plant microbiomes

Assemblages of microbes living in, on and around plants

Endophytic and rhizosphere microbiome

- The rhizosphere and endophytic microbiomes ensure plant health.
- DNA extracted from microbes in:

1. the seed,
2. rhizosphere, and
3. endophytic compartments, and
4. soils.

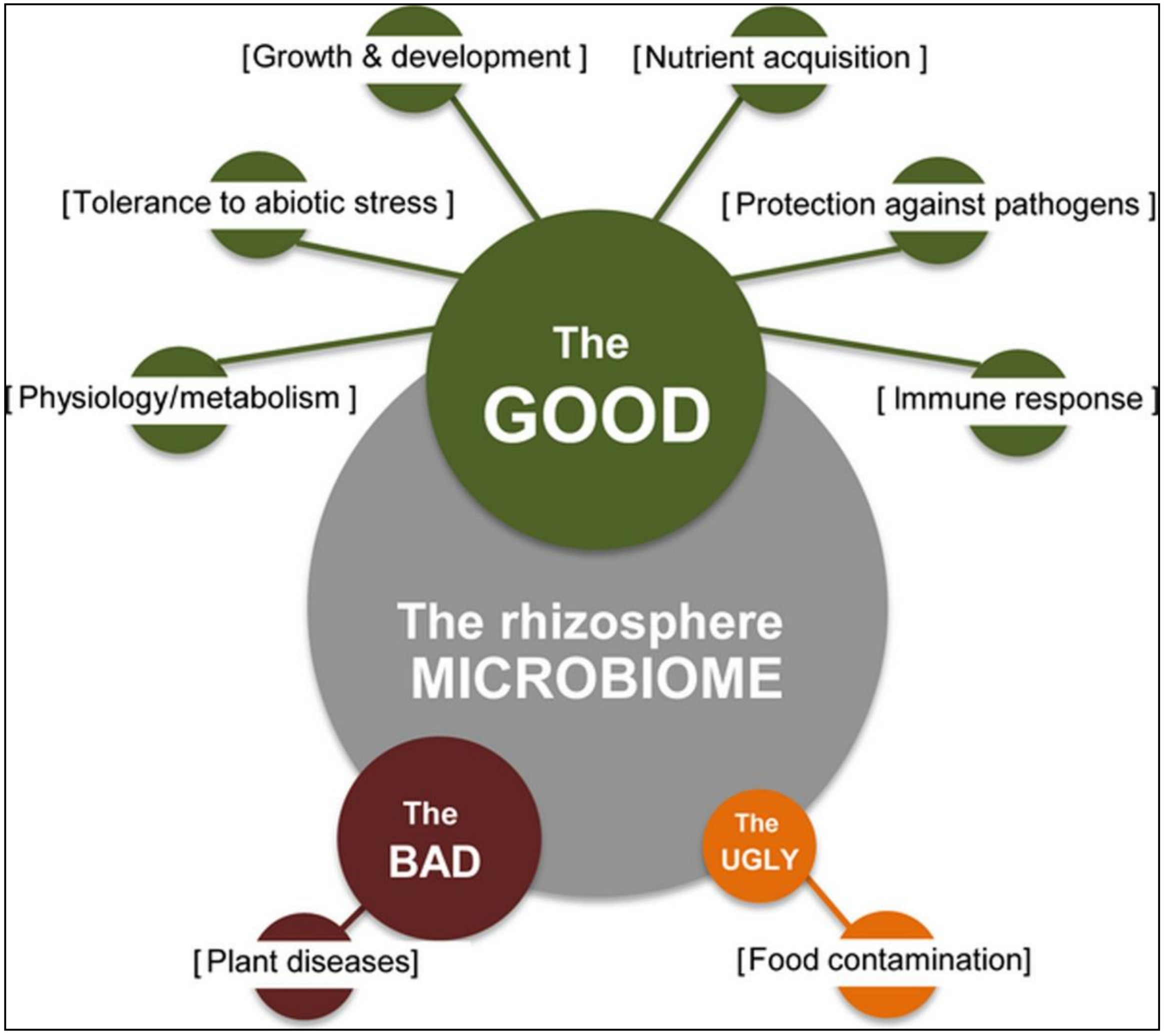


Hirsch & Mauchline,2012; Leach,2015; Johnston-Montje *et al.*,2014;..

Plant microbiomes

Assemblages of microbes living in, on and around plants

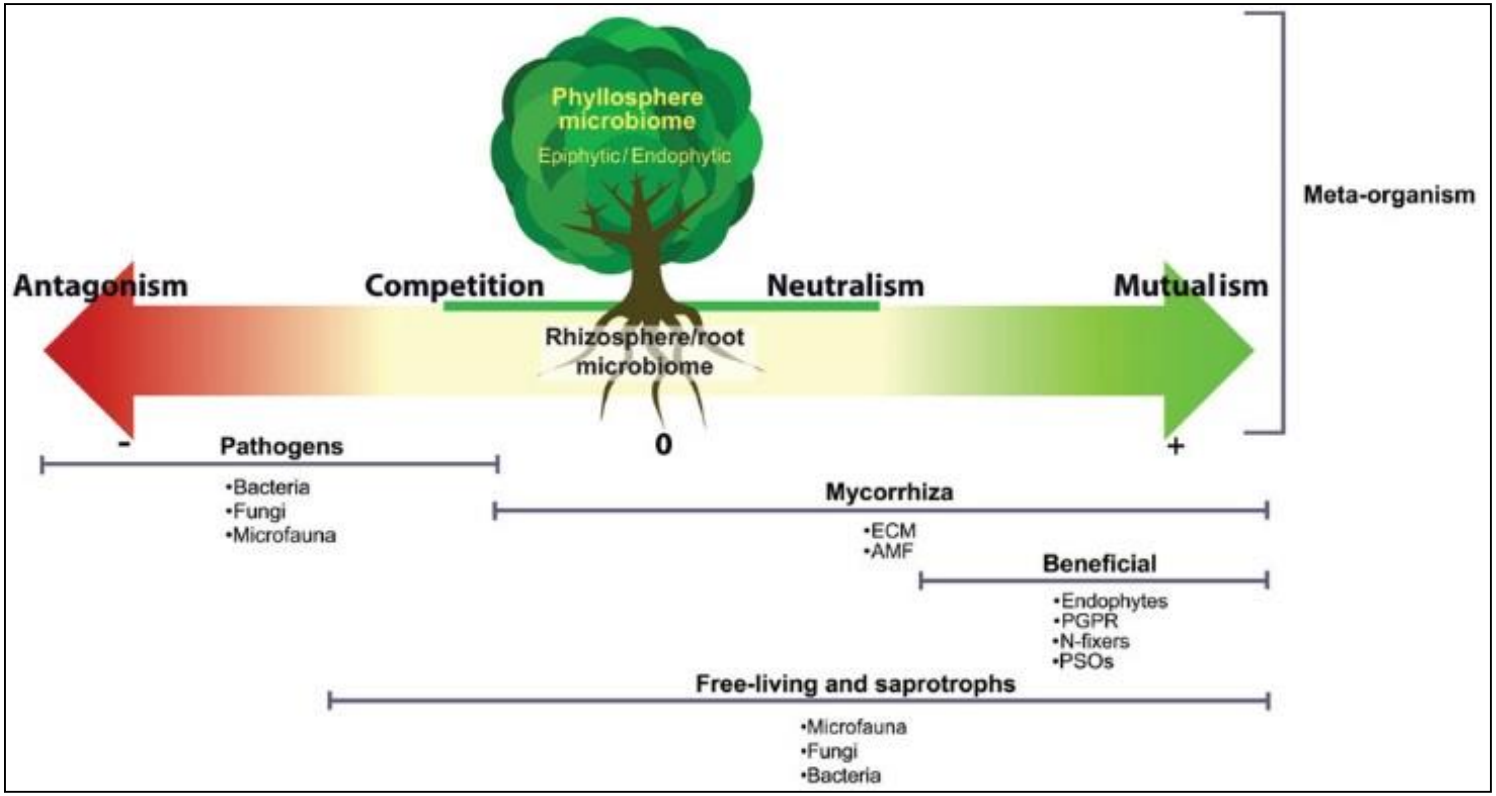
The rhizosphere microbiome



Plant microbiomes

Assemblages of microbes living in, on and around plants

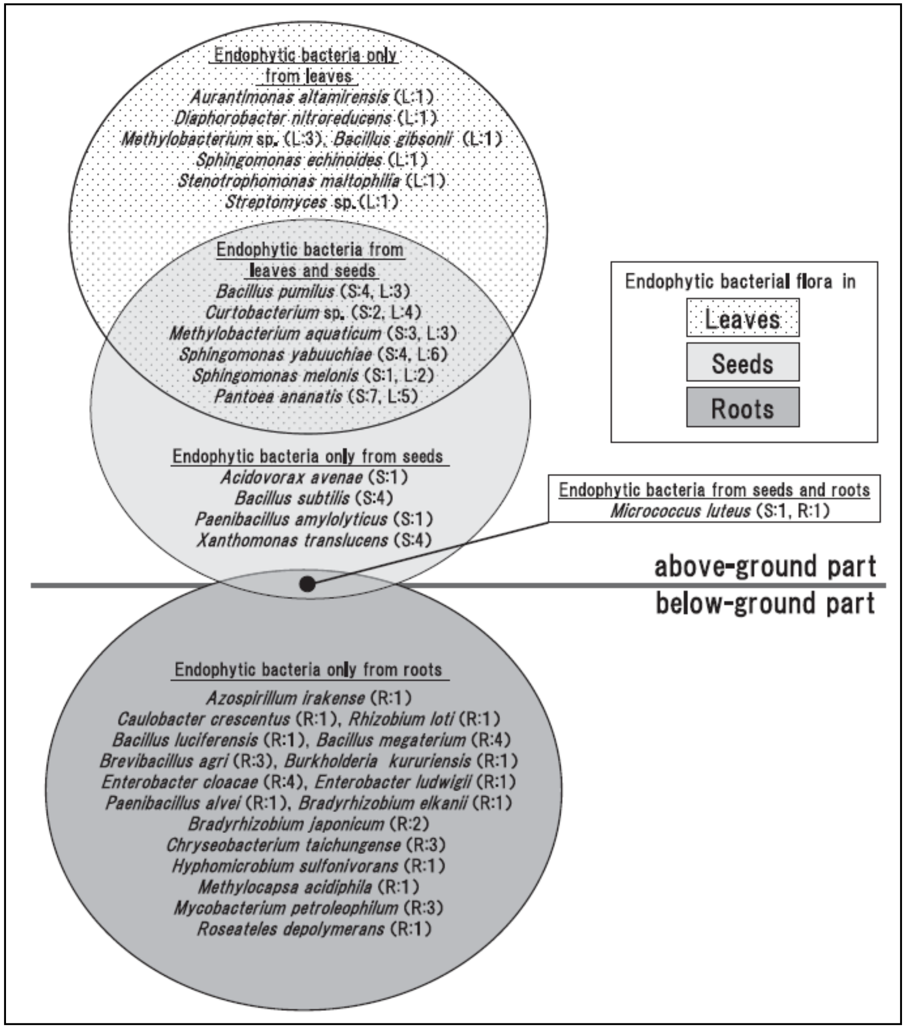
The rhizosphere microbiome



Endophytic bacterial flora

Leaves, seeds and roots of rice plants

- Comparison of the culturable endophytic bacterial floras in leaves, seeds and roots of rice plants(*Oryzae sativa*).
- About 30 strains were isolated from each part of the plant and identified by 16S rRNA gene sequences.



Mano and Morisaki,2008

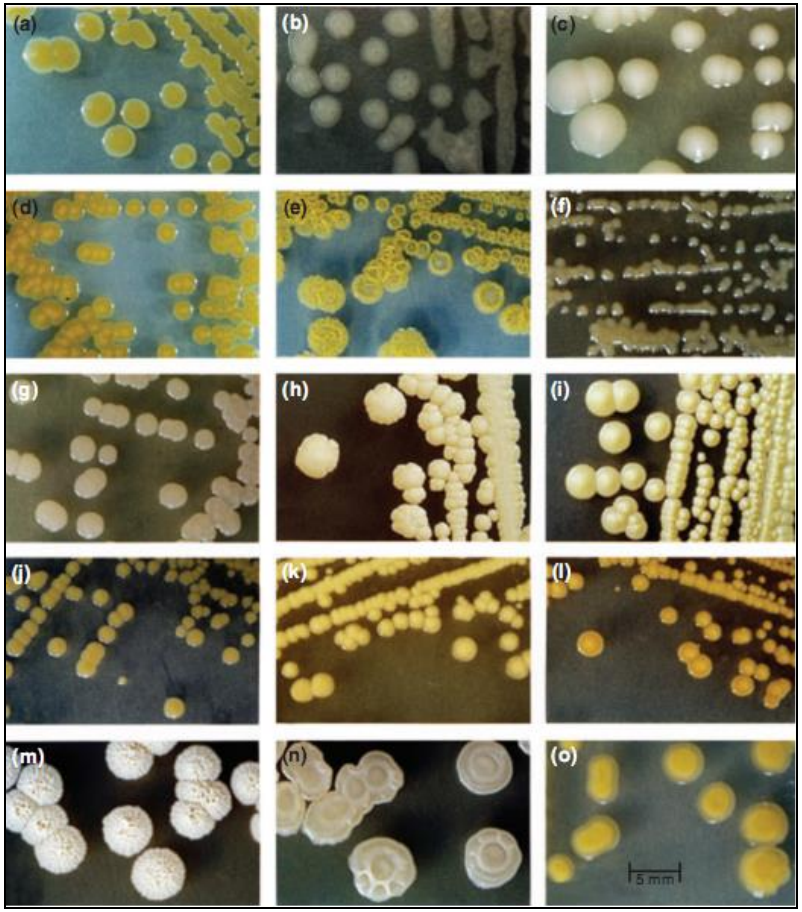
Surface and endophytic bacterial flora
Seeds of rice plants

- The predominant bacteria on and inside of matured rice seeds:
- **Endophytic bacteria:**
- *Acidovorax*
- *Paenibacillus*
- *Pantoea*
- **Surface bacteria:**
- *Stenotrophomonas maltophilia*
- *Agrobacterium*
- **Both inside and outside of the seeds:**
- *Curtobacterium*
- *Methylobacterium*
- *Bacillus*
- *Micrococcus*
- *Xanthomonas*
- *Sphingomonas*

Mano *et al.*,2006

Endophytic rice seed-associated bacteria
Their role in pathogenicity and biological control

- (a) *Pantoea stewartii* LMG 20115, (b) *Pantoea dispersa* LMG 20116, (c) *Enterobacter cloacae* LMG 20117,(d) *Pseudomonas aeruginosa* LMG 20125, (e) *Pseudomonas oryzihabitans* LMG 20126, (f) *Xanthomonas* sp. LMG 20137, (g) *Burkholderia glumae* LMG 20138, (h) *Staphylococcus gallinarum* LMG 20176,(i) *Micrococcus luteus* LMG 20178, (j) *Curtobacterium flaccumfaciens* LMG 20194, (k) *Clavibacter michiganense* LMG 20187, (l) *Cellulomonas flavigena* LMG 20188, (m) *Bacillus pumilus* LMG 20162, (n) *Bacillus subtilis* LMG 20163 and (o) *Paenibacillus polymyxa* LMG 20164.



Cottyn *et al.*,2009

Plant microbiomes

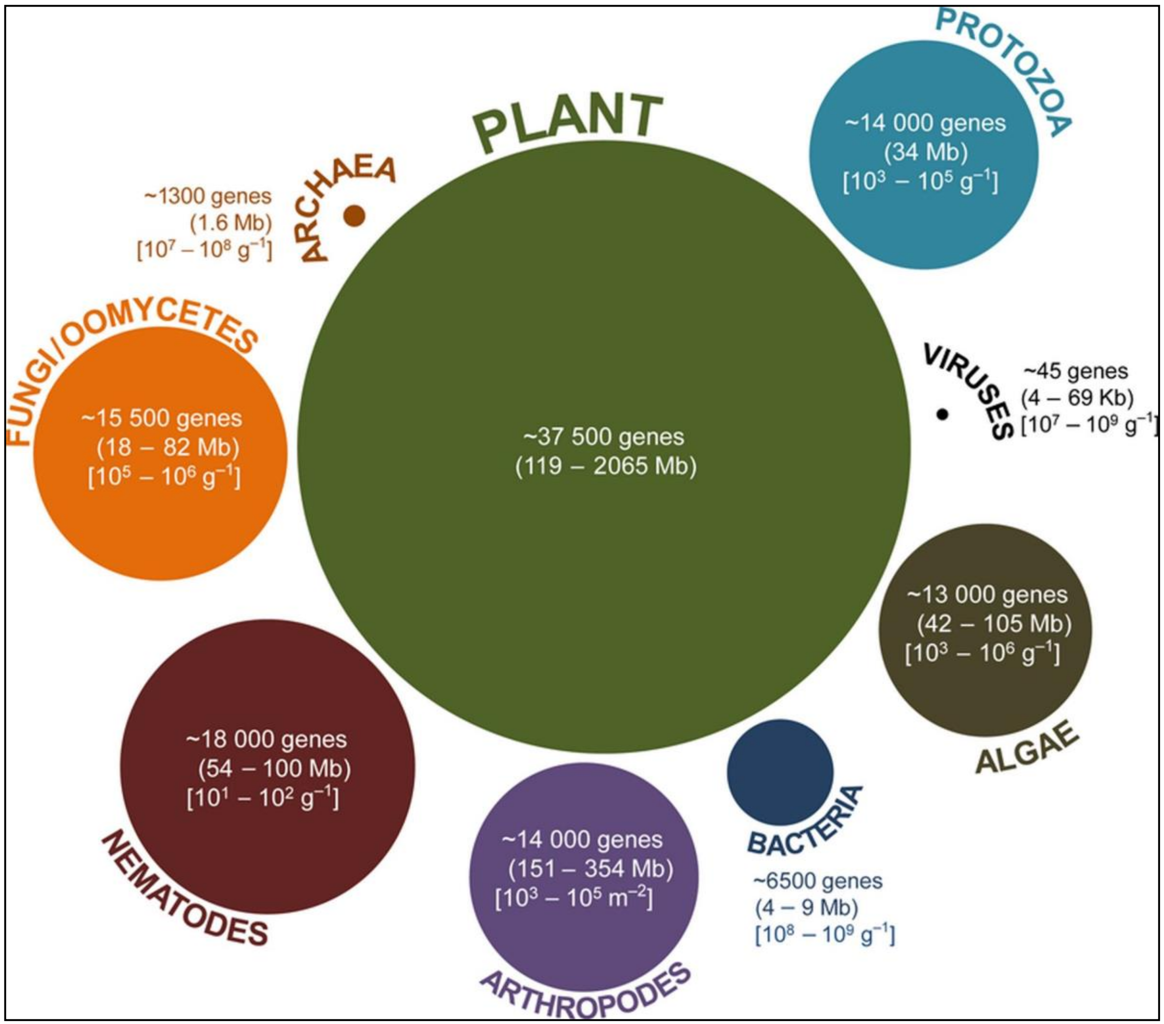
Abundance and genome size of (micro) organisms present in the rhizosphere zoo

- The circle's size, except for VIRUSES, is a measure of the average number of genes in the genomes of representative species of each group of organisms; the size (or size range) of their respective genomes is indicated between parentheses.
- For each of these (micro) organisms, the approximate numbers for their abundance are indicated between square brackets.

Mendes *et al.*,2013

Plant microbiomes

Overview of (micro) organisms present in the rhizosphere zoo



Genome size

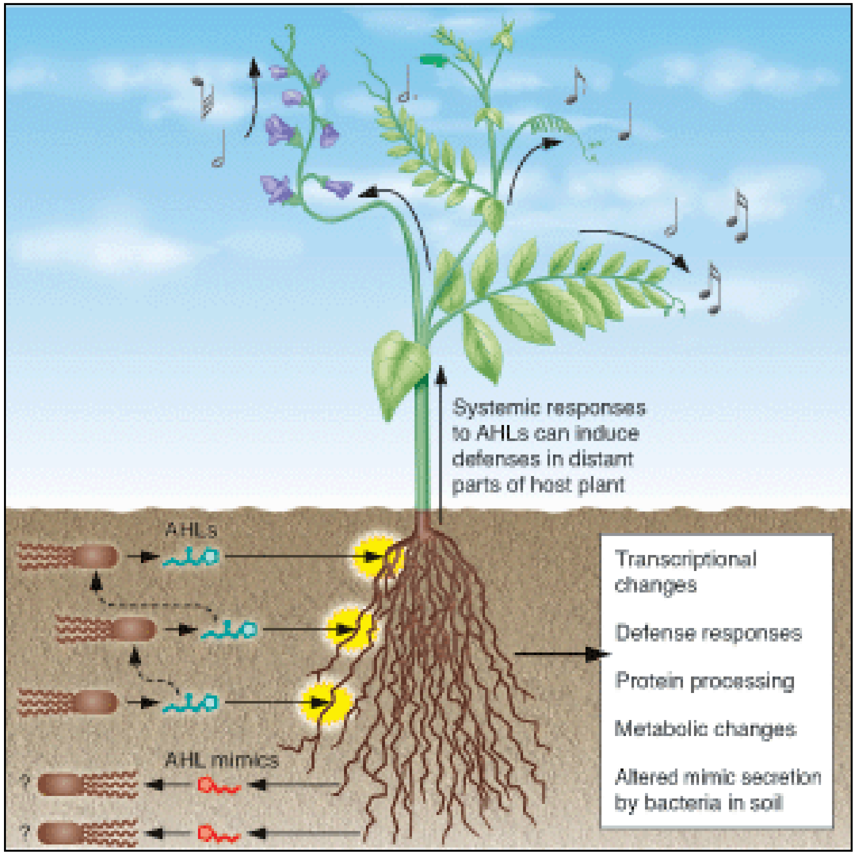
Comparison of genome size in different organisms

Organism type	Organism	Genome size (base pairs)		Approx. no. of genes	Note
Virus	*Pandoravirus salinus*	2,470,000	2.47Mb		Largest known viral genome.
Virus	Megavirus	1,259,197	1.3Mb		Until 2013 the largest known viral genome.
Virus	Bacteriophage MS2	3,569	3.5kb		First sequenced RNA-genome
Plant	*Paris japonica* (Japanese-native, pale-petal)	150,000,000,000	150Gb		Largest plant genome known
Plant	Genlisea tuberosa	61,000,000	61Mb		Smallest recorded flowering plant genome, 2014.
Plant	Arabidopsis thaliana	135,000,000	135 Mb	27,655	First plant genome sequenced, December 2000.
Nematode	Pratylenchus coffeae	20,000,000	20Mb		Smallest animal genome known
Mammal	Homo sapiens	3,289,000,000	3.3Gb	20000	Homo sapiens estimated genome size 3.2 billion bp[Initial sequencing and analysis of the human genome
Mammal	Bonobo	3,286,640,000	3.3Gb	20000	Pan paniscus estimated genome size 3.29 billion bp
Insect	Drosophila melanogaster (fruit fly)	175,000,000	175Mb	13600	Size variation based on strain (175-180Mb; standard y w strain is 175Mb)
Insect	Solenopsis invicta (fire ant)	480,000,000	480Mb	16569	
Fungus-yeast	Saccharomyces cerevisiae	12,100,000	12.1Mb	6294	First eukaryotic genome sequenced, 1996
Fungus	Aspergillus nidulans	30,000,000	30Mb	9541	
Bacterium – cyanobacterium	*Prochlorococcus* spp.	1,700,000	1.7Mb	1884	Smallest known cyanobacterium genome
Bacterium	*Haemophilus influenzae*	1,830,000	1.8Mb		First genome of a living organism sequenced, July 1995
Bacterium	*Escherichia coli*	4,600,000	4.6Mb	4288	

Wikipedia,2017

Quorum sensing
Inter-kingdom signalling

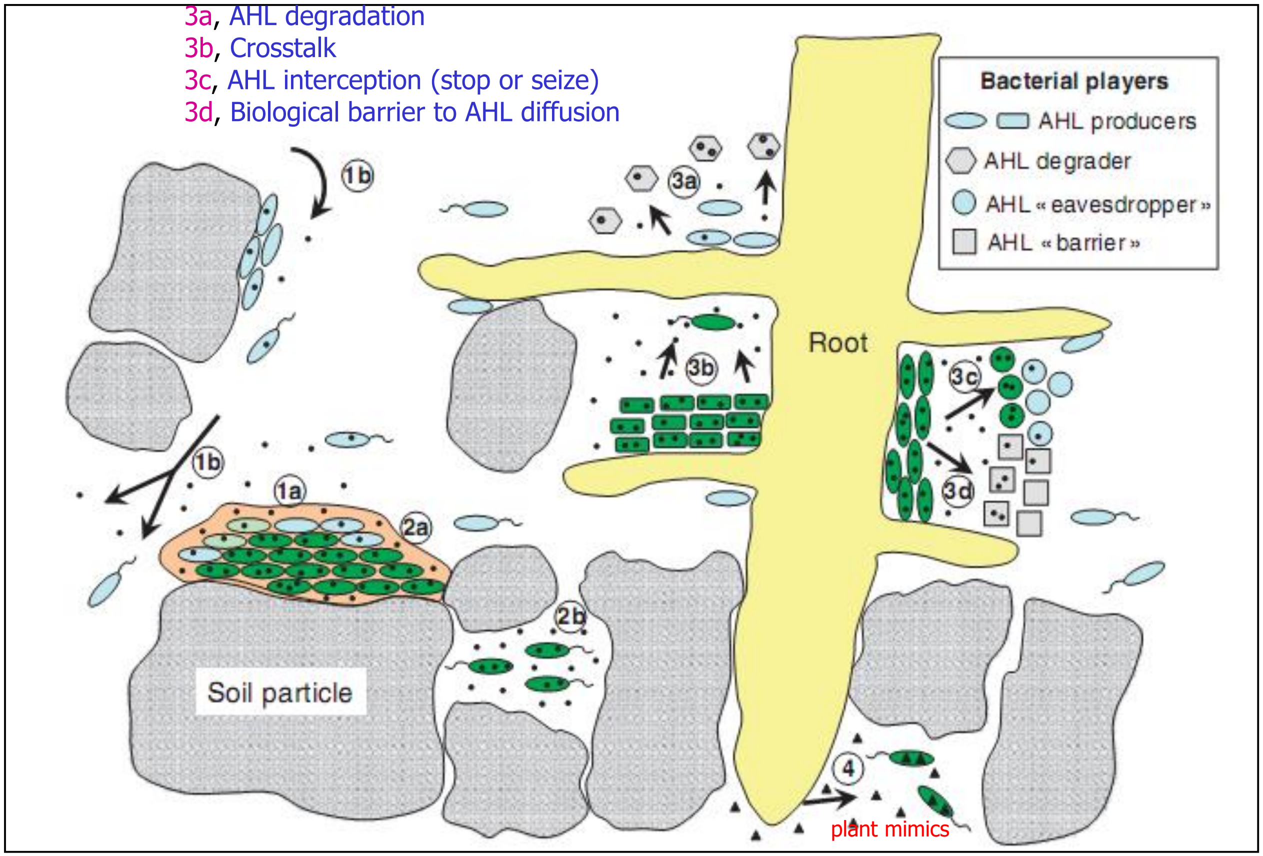
- Music to its ears: An eukaryotic host listens to bacterial AHL conversations.
- AHLs produced by associated bacteria are detected by both the host and the bacteria.
- The host responds by altering many functions, including defenses, metabolism and production of AHL mimic compounds.
- Systemic responses to the bacterial AHLs can induce defenses in distant parts of the host.



Bauer *et al.*,2005

How bacteria communicate among their own kind and with other species

Abundance of microbial signaling in rhizosphere zoo



AHL (small dark circles) and plant mimics (small dark triangles) are represented. Light blue cells do not 'quorate' (not having a quorum) whereas green cells 'quorate'.

Boyer and Wisniewski-Dyé,2009

Plant microbiomes

Assemblages of microbes living in, on and around plants

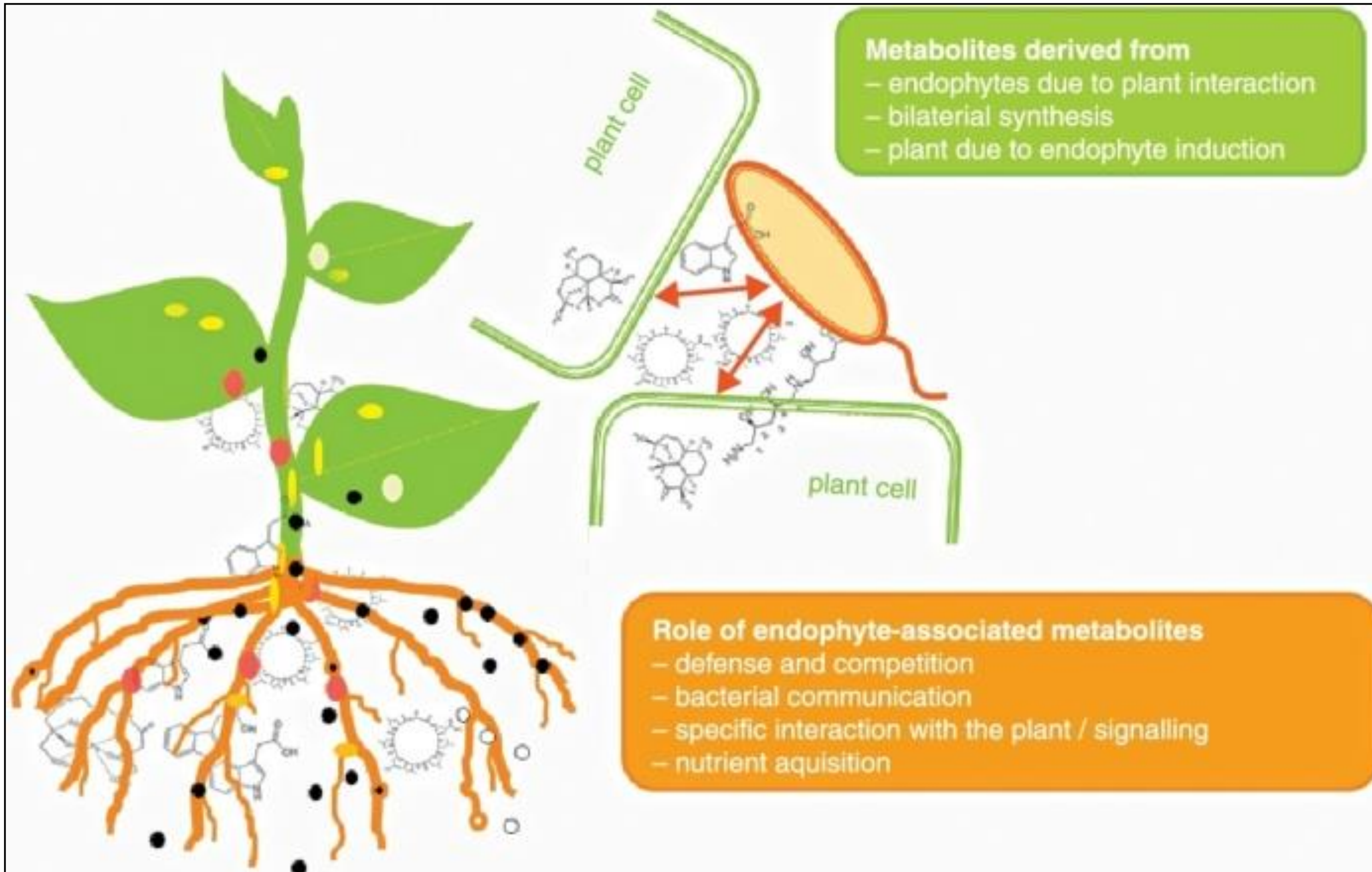
Endophytic and rhizosphere microbiome

1. What microbes are in and on plant surfaces (usually leaf)?
2. What factors are important for their survival, colonization, and distribution on the surface? their ability to cause disease or induce resistance?
3. How do they communicate with each other and with the plants?

Plant microbiomes

Assemblages of microbes living in, on and around plants

Metabolic potential of endophytic bacteria



Brader,2014

The rhizosphere microbiome
Interactions in the rhizosphere
Microbiome to the rescue

- Infected plants perceive pathogen invasion in roots or shoot and subsequently increase the secretion of microbe-stimulatory compounds in non-infected roots.
- These stimulants can recruit and activate plant-beneficial microorganisms.
- Beneficial microorganisms can:

1. Induce resistance (IR) directly, or
2. Produce pathogen-inhibitory compounds.

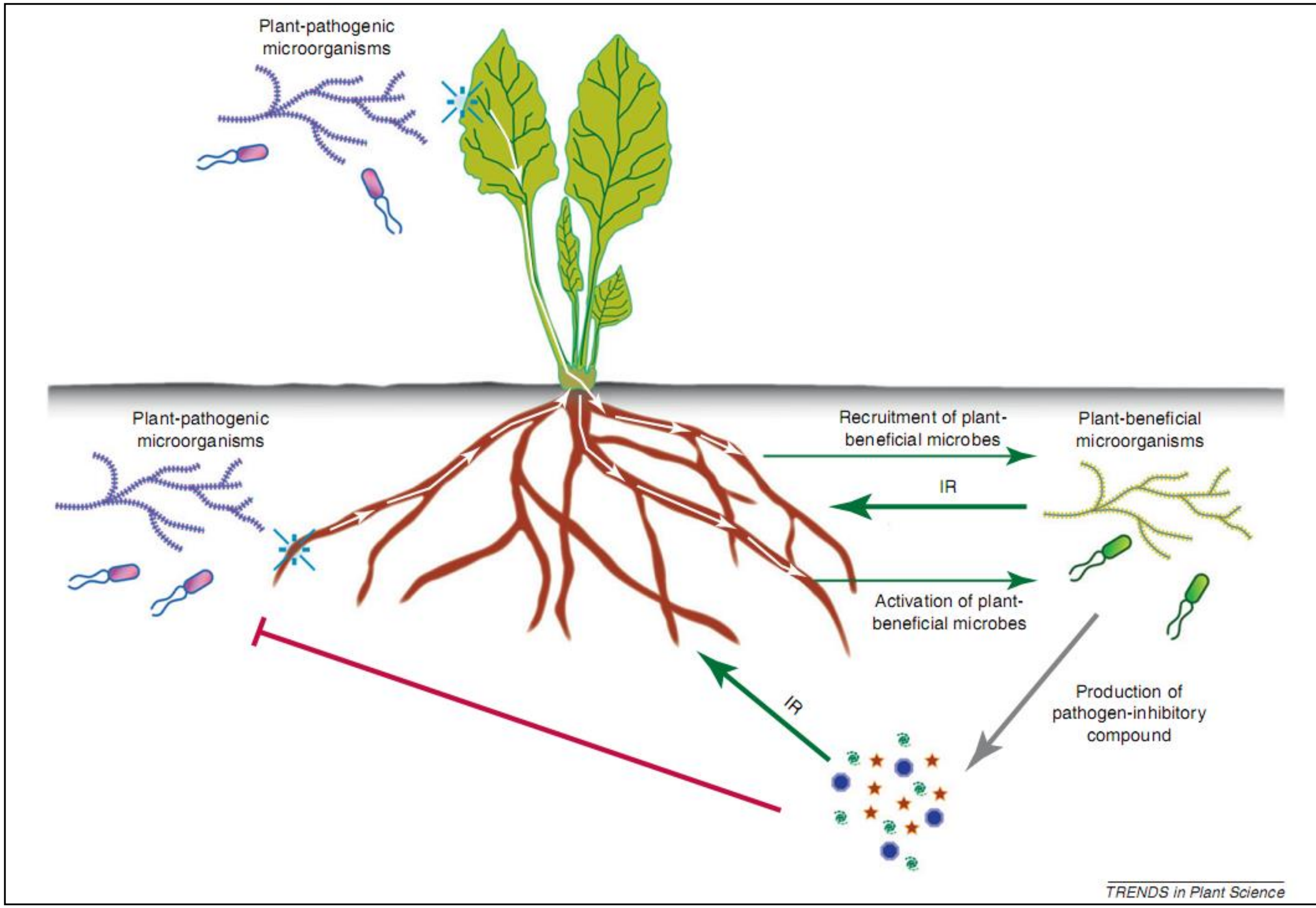
- Some pathogen-inhibitory compounds are known to induce resistance themselves.

Berendsen *et al.*,2012

The rhizosphere microbiome

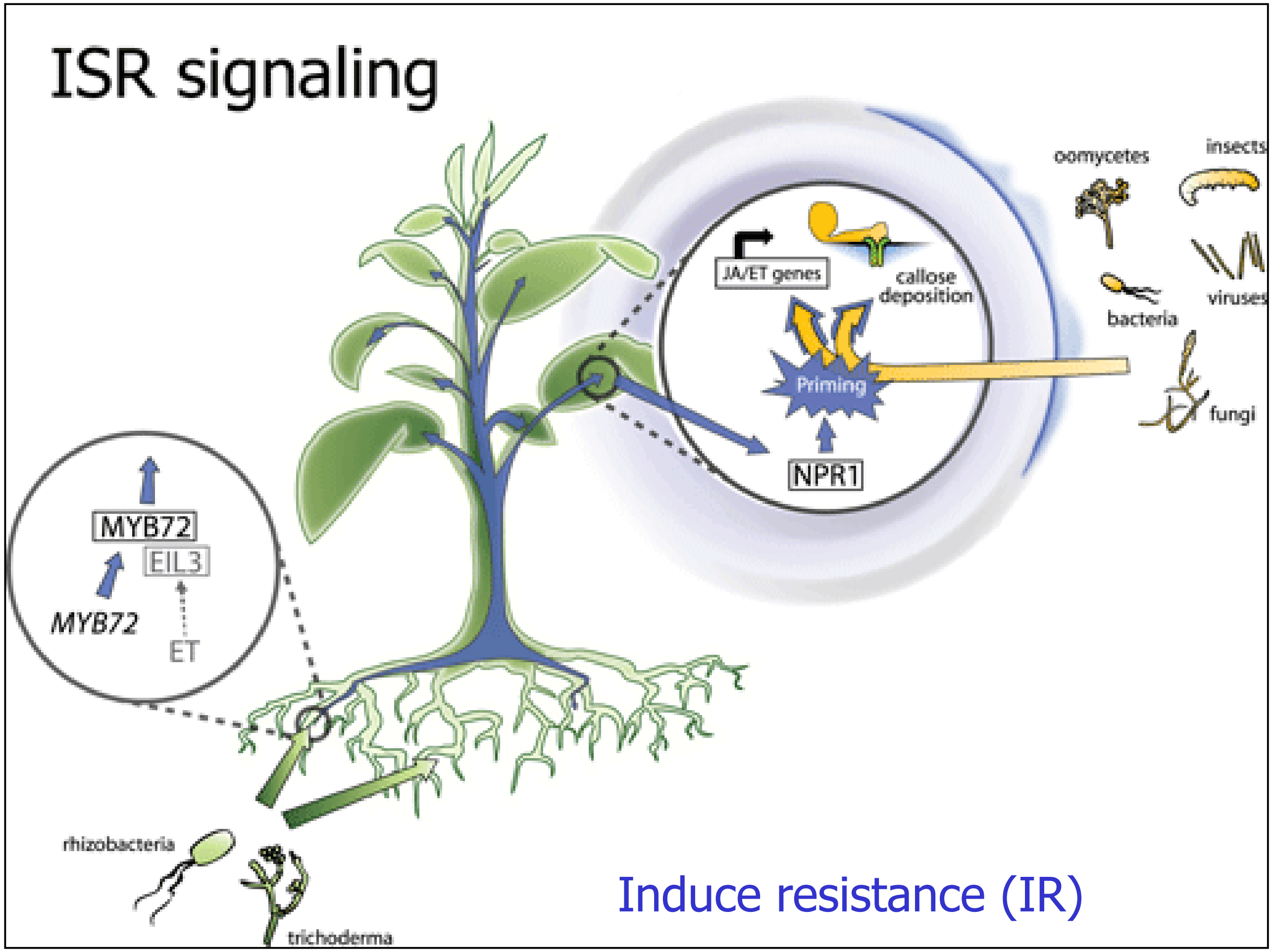
Interactions in the rhizosphere

Microbiome to the rescue



Berendsen *et al.*,2012

The rhizosphere microbiome
Interactions in the rhizosphere
Microbiome to the rescue



Phytobiomes (probiotic)

Plant probiotics

Plant probiotic microbes(bacteria, yeasts,..)

- In natural environments, the nutrients uptake of plants, as well as their health, is greatly regulated by the presence and activity of beneficial micro-organisms which are now known as plant probiotics.
- Beneficial microbes are known to enhance plant growth:

1. By fixing atmospheric nitrogen, solubilizing phosphorus, nitrogen, iron and other nutrients,
2. by producing bioactive compounds that stimulate root proliferation, and
3. by suppressing root diseases.

Bosco and Picard,2008

The rhizosphere microbiome

Interactions in the rhizosphere

Plant probiotic microbes (bacteria, yeasts,..)

- These beneficial micro-organisms are now called plant probiotics (Picard and Bosco,2007), and include:

1. mycorrhizal fungi,
2. antagonistic fungi, and
3. the large group of Plant Growth Promoting Rhizobacteria (PGPR).

Most soil and plant scientists feel that the well known term PGPB and PGPR is simple and informative enough.
Earlier, it was plain biofertilizers, then bioinoculant arrived and now slowly plant probiotic.

Bosco and Picard,2008;...

Ectomycorrhizal symbiosis
Mycorrhizal fungi
Mycorrhiza Helper Bacteria

- Some soil bacteria have been shown to have beneficial effects upon the establishment of ectomycorrhizal symbioses.
- Some of these bacteria, known as Mycorrhiza Helper Bacteria (MHBs), have been shown to stimulate ectomycorrhiza formation, root and shoot biomass. E.g.
- *Arthrobacter*
- *Azospirillum brasilense*
- *Azotobacter*
- *Bacillus*
- *Burkholderia*
- *Paenibacillus*
- *Pseudomonas*
- *Streptomyces*
- *Klebsiella*

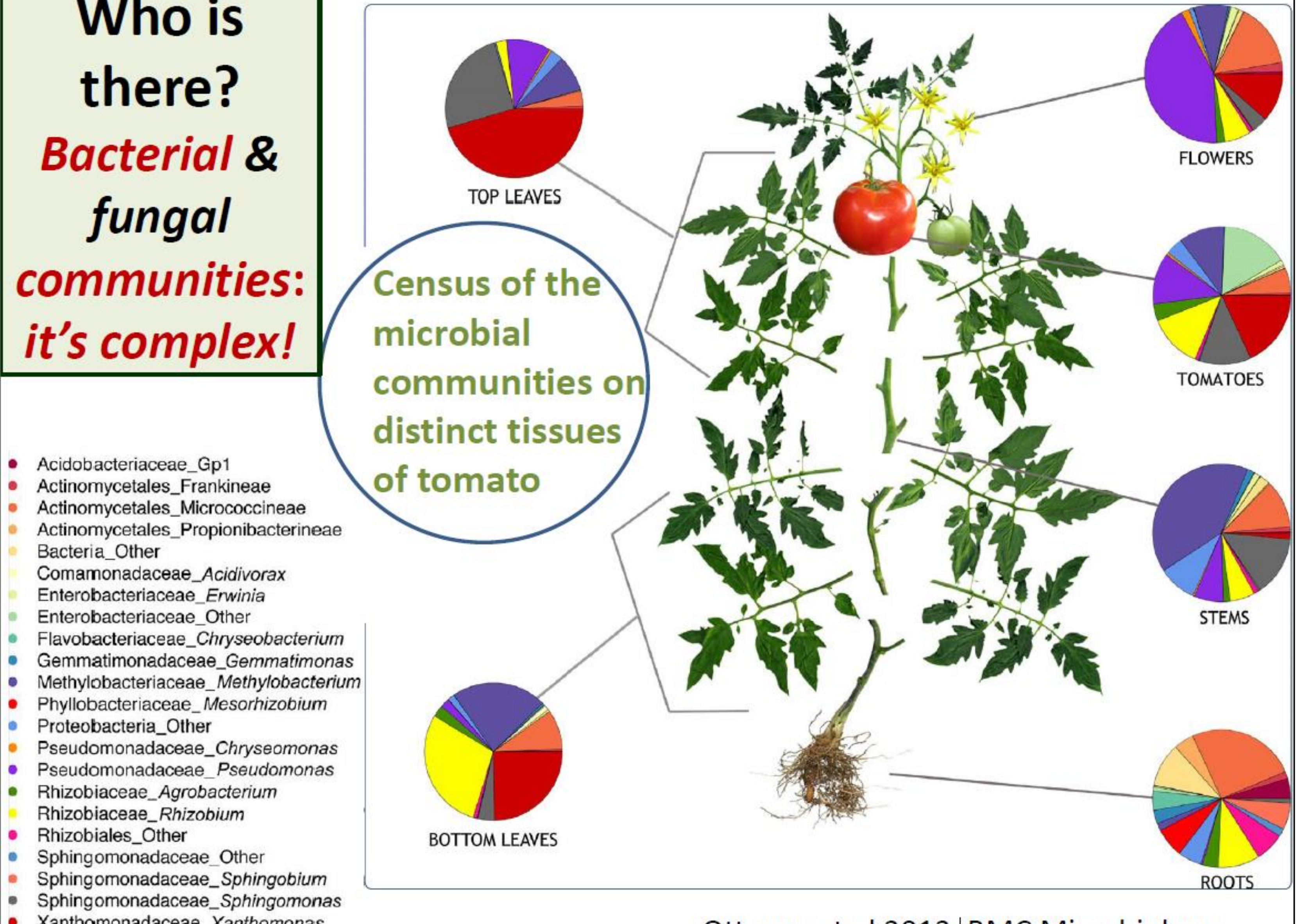
Frey-Klett *et al*.,2007

The rhizosphere microbiome and plant health
Interactions in the rhizosphere
Plant probiotics

- Several model organisms for plant growth promotion and plant disease inhibition are well-studied including:
- The bacterial genera:
- *Azospirillum*
- *Rhizobium*
- *Serratia*
- *Bacillus*
- *Pseudomonas*
- *Stenotrophomonas*
- *Streptomyces*
- The fungal genera:
- *Ampelomyces*, *Coniothyrium*, and *Trichoderma*.

Song *et al*.,2012

Who is there? *Bacterial* & *fungal* *communities*: *it's complex!*



Ottesen et al 2013. BMC Microbiology

Metagenome
Environmental genome

- Metagenomics:
- Meta (meaning "after", or "beyond"). A prefix used in to indicate a concept which is an abstraction behind another concept, used to complete or add to the latter.
- Genomics- an interdisciplinary field of science focusing on genomes.

The term "metagenomics" was first used by Jo Handelsman, Jon Clardy, Robert M. Goodman, Sean F. Brady, and others, and first appeared in publication in 1998.

Metagenome

Environmental genome

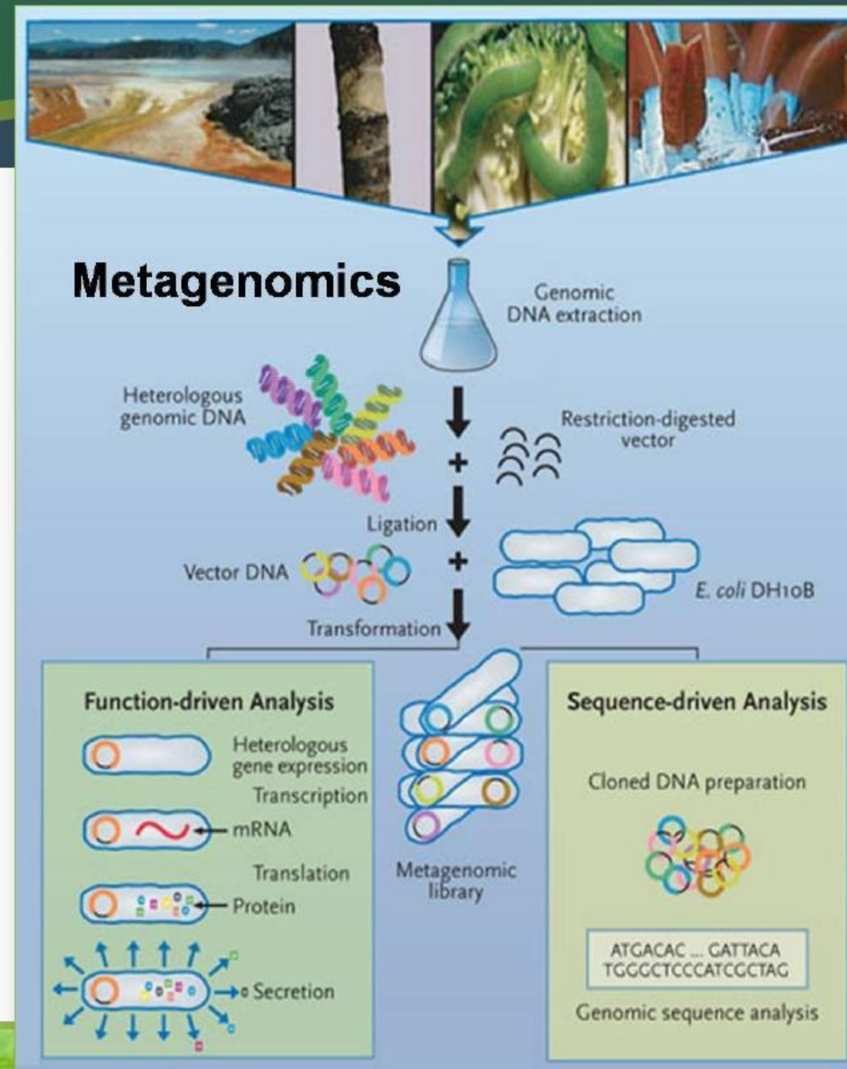
- Metagenomics, in contrast to Metataxonomics (16S rRNA analysis), is the study of the entire genetic content of all microbiota members in a natural habitat by utilization of the whole genome sequencing technique.
- The field centers upon direct genetic analysis of microbial genomes isolated from various environments ranging from the human gut to geothermal hot springs.
- Metagenomics is an alternative approach to targeted amplicon sequencing in the study of uncultured microbiota.

APS

Enter:

METAGENOMICS!

- Genomic study of <u>all</u> organisms
  - Sequencing 16S rRNA, DNA, or mRNA from environmental samples
- Address questions on:
  - community composition (*“Who is there?”*)
  - function (*“What can they do?”*)
  - activity (*“What are they doing?”*)



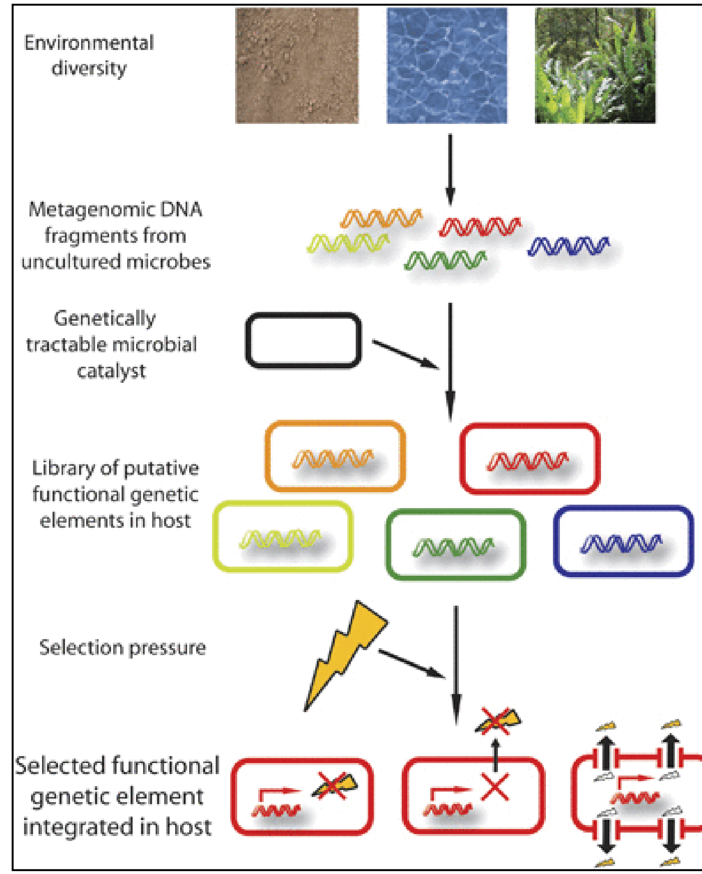
Metagenomics is the study of genetic material recovered directly from environmental samples.

Jan E. Leach

Metagenomics

Environmental genomics, ecogenomics or community genomics

- Metagenomics is the study of genetic material recovered directly from environmental samples.
- The broad field may also be referred to as:
- environmental genomics,
- Ecogenomics, or
- community genomics.



Wikipedai,2017

Specific aims of a metagenomics

- By offering direct access to the entire genetic make-up of microbial communities, metagenomics can provide valuable molecular insights into novel enzymes and biocatalysts, as well as into genomic linkages between community function and structure.
- The metagenomics approach serves as a powerful tool for elucidating the relationship between host-associated microbial communities and host phenotype.

Specific aims of a metagenomics

- Diversity patterns of microorganisms can be used for monitoring and predicting environmental conditions and change.
- Examining genes/operons for desirable enzyme candidates (e.g., cellulases, chitinases, lipases, antibiotics, other natural products). These may be exploited for industrial or medical applications.
- $2.3 billion in sales of industrial enzymes in 2003.
- Discovery of novel enzymes and catalysts with industrial uses by screening thousands of microbial species simultaneously.
- Look for pharmacologically interesting genes (e.g. antibiotics) that exist in organisms that cannot be cultured.
- Examining secretory, regulatory, and signal transduction mechanisms associated with samples or genes of interest.

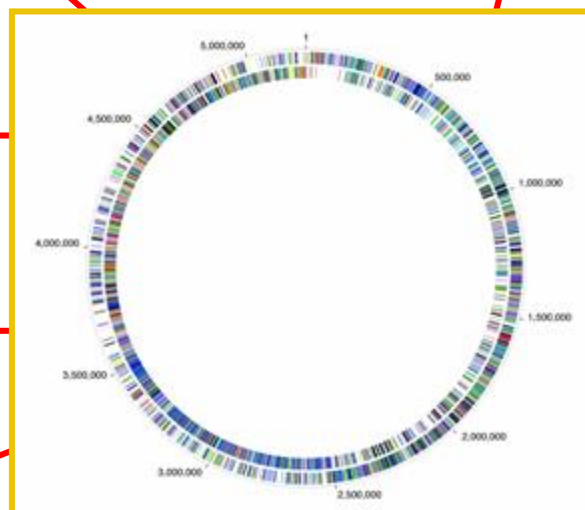
Specific aims of a metagenomics

Understanding Cell Structure & Function

Understanding Host Interactions

Understanding Expression (RNA/Protein)

Understanding Metabolism



Discover DNA Variation, Genotyping Forensics

Genome Engineering

Drug/Vaccine Development

Understanding Protein-Protein Interactions

Defining the Minimal Gene Set

TIGR: The Institute for Genomic Research

The J. Craig Venter Institute

Specific aims of a metagenomics

What are the advantages of metagenome sequencing?

- Metagenomics sequencing can potentially play a significant role in quality control, for example as a water quality assessment tool.
- Independence from taxonomically informative genetic markers.
- Ability to study highly diverged microbes, such as viruses.
- Close estimations of microbial diversity.
- Detection of abundance of microorganisms in various environments.
- Analysis of unculturable microorganisms.
- Information on composition as well as functional capabilities of an ecosystem.
- Investigation of function genes and gene clusters.

GATC Biotech AG.,2017

Analysis of Metagenomics Data

How do we assess microbiomes?

- Metagenomes are big
  - Soil has as many as 40,000 individual microbial species
  - Soil metagenome orders of magnitude bigger than human genome
- Analyzing the metagenome
  - Screens
  - Phylogenetic studies
  - Sequencing uncultivated organisms
  - Studying metagenome under different conditions.

Specific aims of a metagenomics

Difference between genomics and metagenomics and 16S rRNA sequencing

- The main difference between genomics and metagenomics is the nature of the sample.
- Genomics explores the complete genetic information of a single organism only, whereas metagenomics explores a mixture of DNA from multiple organisms and entities, such as viruses, viroids and free DNA.
- Amplicon sequencing, most frequently of the 16S rRNA or ITS genes, involves DNA extraction from all cells in a sample and then the targeting of a taxonomically informative genomic marker that is common to a specific group of interest.

GATC Biotech AG.,2017

Specific aims of a metagenomics

Difference between genomics and metagenomics and 16S rRNA sequencing

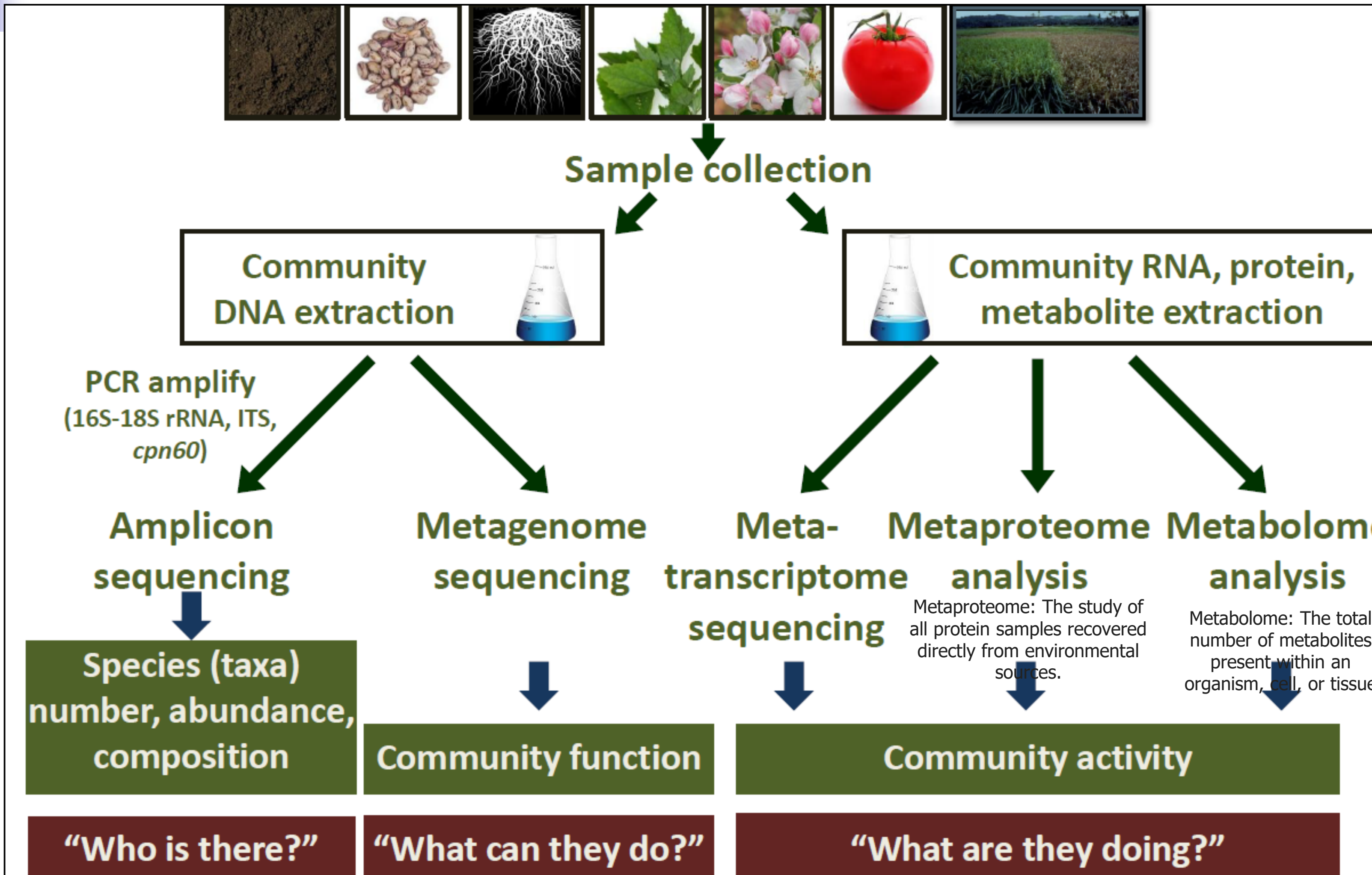
- The resultant amplicons are sequenced and bioinformatically assessed to determine which microorganisms are present in the sample and in what relative abundance.

  In metagenomic next-generation sequencing, DNA is again extracted from all cells in a community. Rather than targeting a specific genomic locus for amplification, all DNA is sheared into fragments and independently sequenced by a shotgun sequencing approach. The resulting reads are aligned to various genomic locations for all genomes present in the sample, including non-microbes.

GATC Biotech AG.,2017

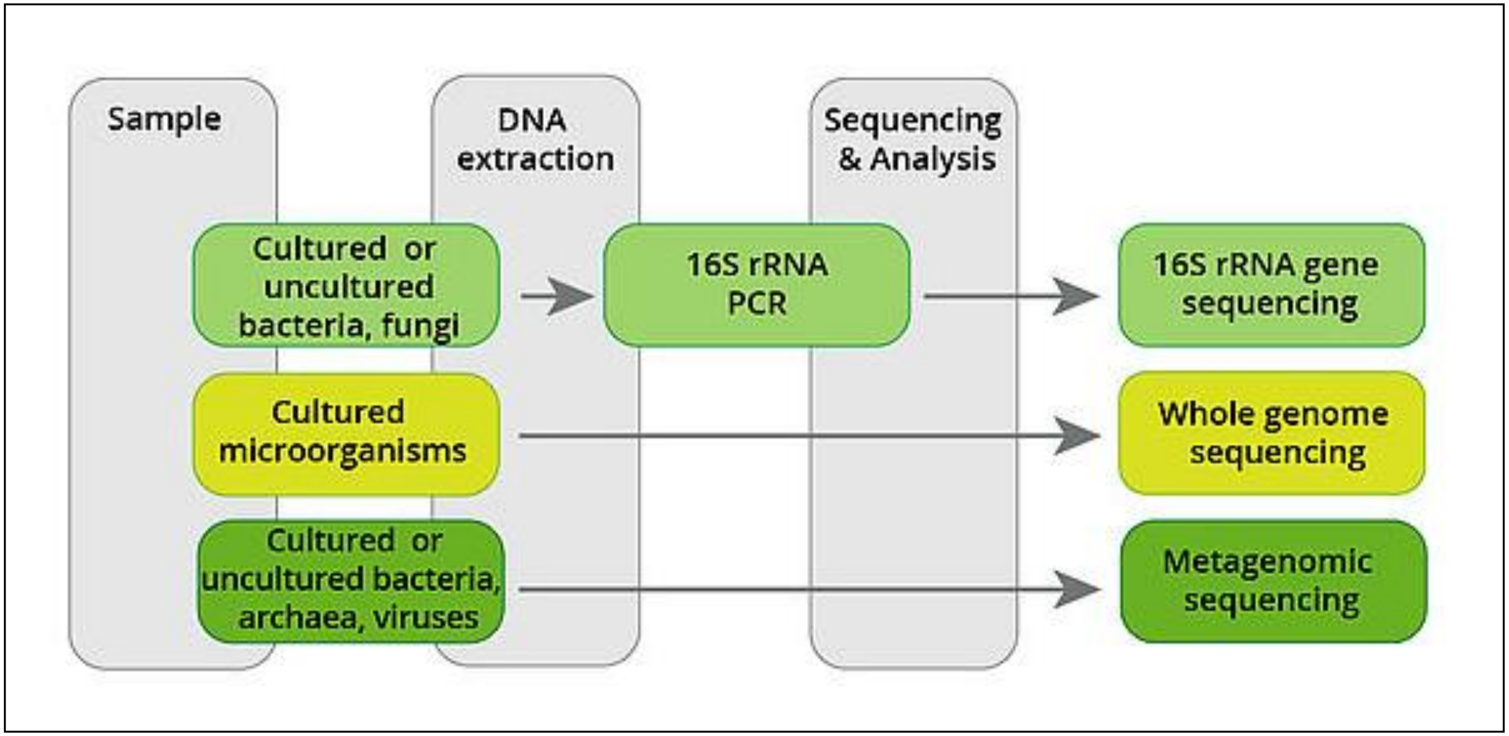
Plant microbiomes

How do we assess microbiomes?



Specific aims of a metagenomics

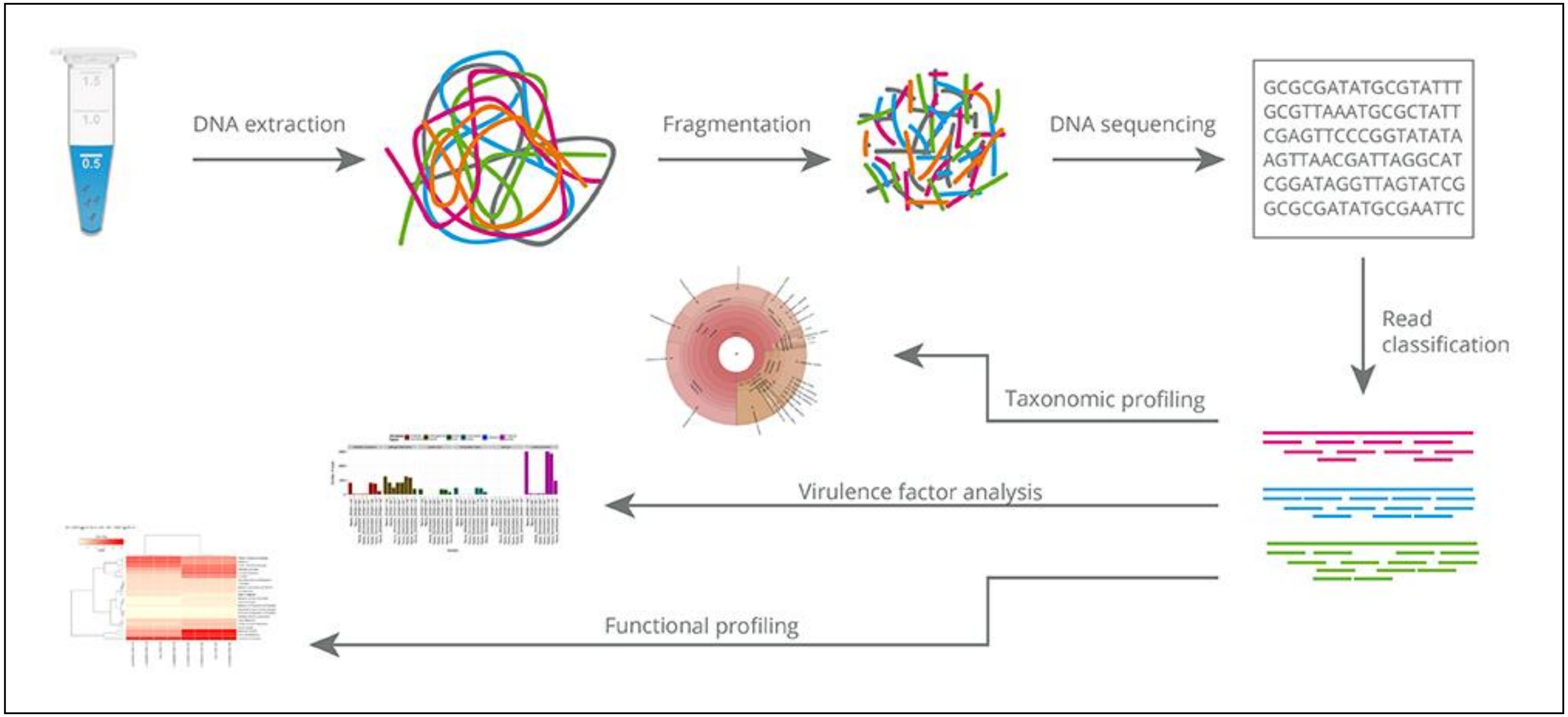
How do we assess microbiomes?



GATC Biotech AG.,2017

Specific aims of a metagenomics

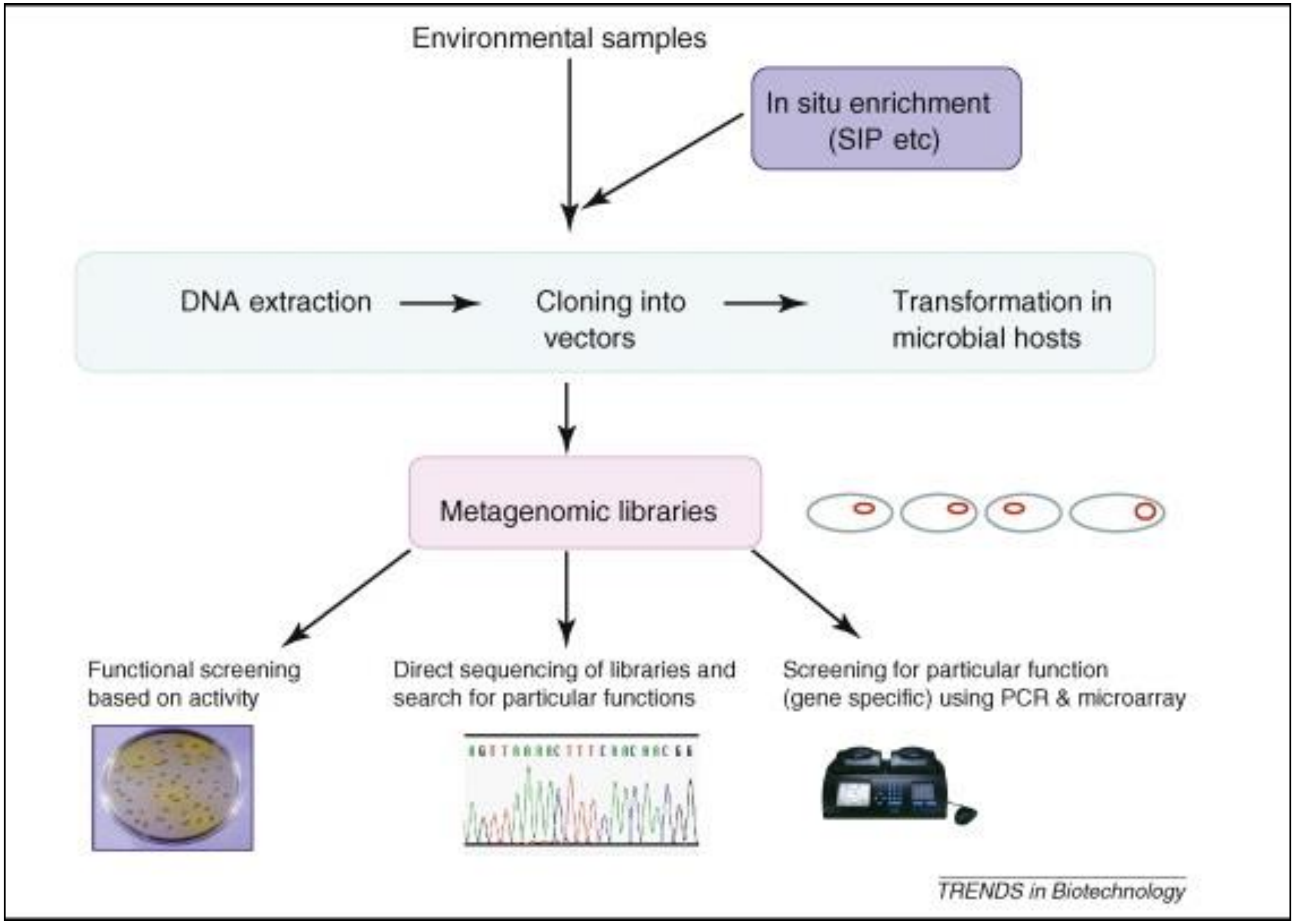
How do we assess microbiomes?



GATC Biotech AG.,2017

Specific aims of a metagenomics

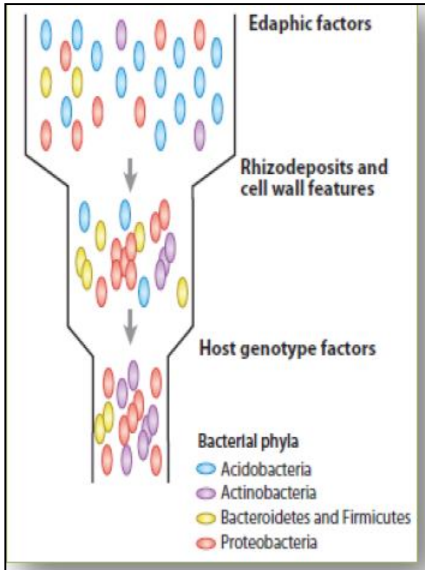
How do we assess microbiomes?



Plant microbiomes

Plants can select microbiome

- Plant genotype – dependent selection fine-tunes the internalized microbial community profiles.
- Plants can transmit bacterial endophytes from generation to generation through seed.



Lundberg Nature 2012
Bulgarelli Nature 2012

Johnston-Montje *et al.*,2014

Plant microbiomes

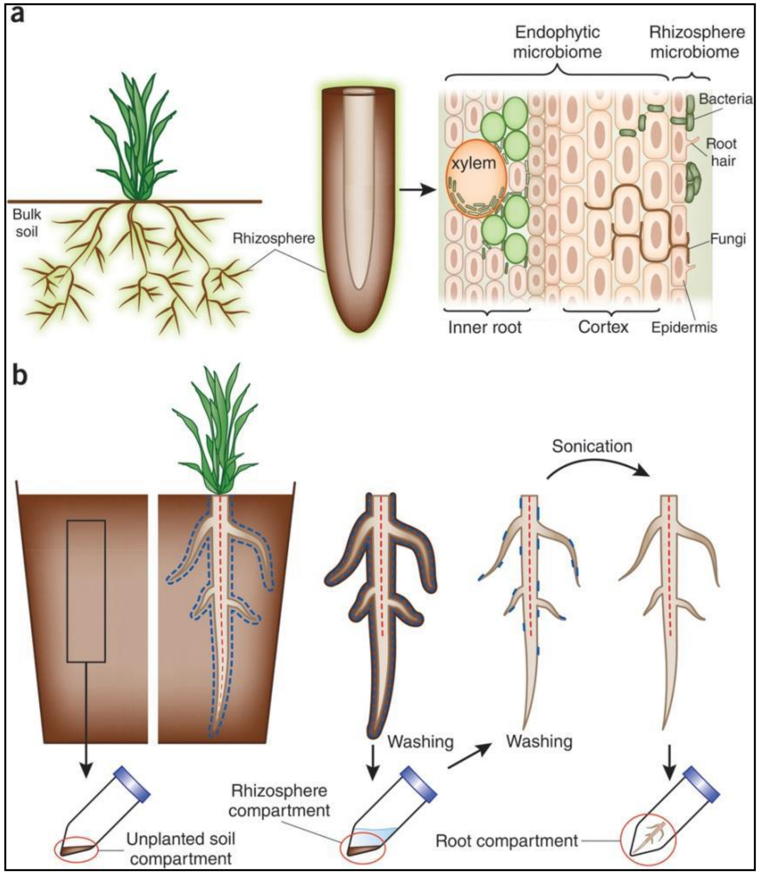
Metagenomic studies

Do plants control their microbiome composition?

- Two recent root metagenomic studies:
- DNA extracted from microbes in:

1. the seed,
2. rhizosphere, and
3. endophytic compartments, and
4. soils.

- amplicon sequencing.



Jan E. Leach; Johnston-Montje *et al.*,2014;..

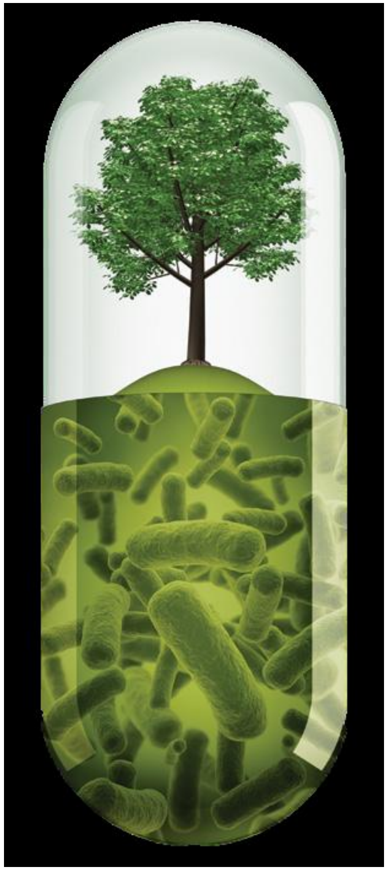
Plant microbiomes
Plants can select microbiome

1. Can we breed plants that select for a beneficial microbiome?
2. Have we inadvertently(accidentally) selected against plant traits that help support beneficial microbes by breeding for high yield under conditions of high inputs and soil tillage?
3. What is the potential for identifying new, more successful biocontrol agents?

Plant microbiomes

The Future

1. Management strategies that create disease-suppressive microbial communities.
2. Plants that select for and maintain beneficial microbiomes.



Brett Ryder

Plant microbiomes

The Future

- Smart microbes that detect and treat disease/destroy pests.

Brett Ryder

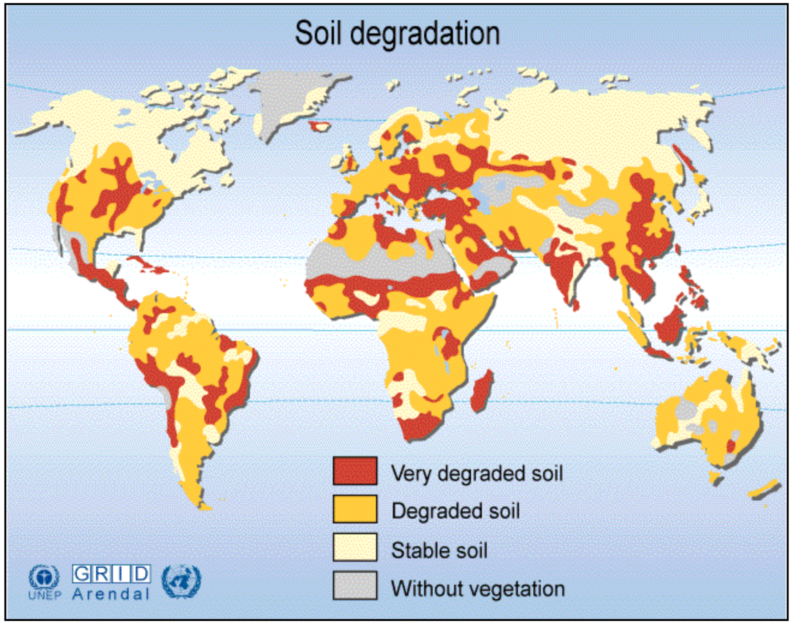
Plant microbiomes

The Future

Managed/engineered microbiomes

- Managed/engineered microbiomes that promote:

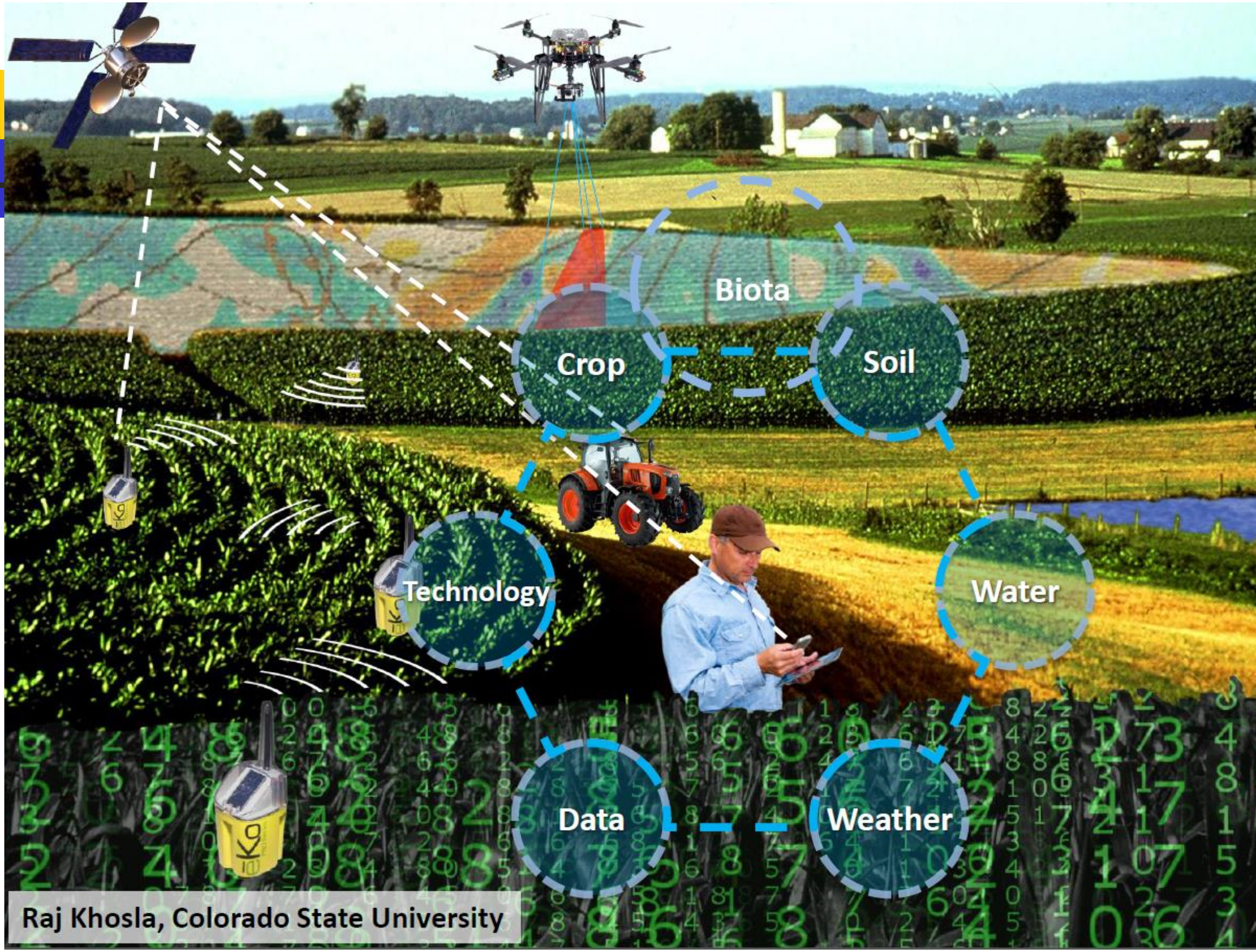
1. sustained crop productivity;
2. rebuild depleted/degraded soils;
3. produce with less water;
4. produce in changing climate.

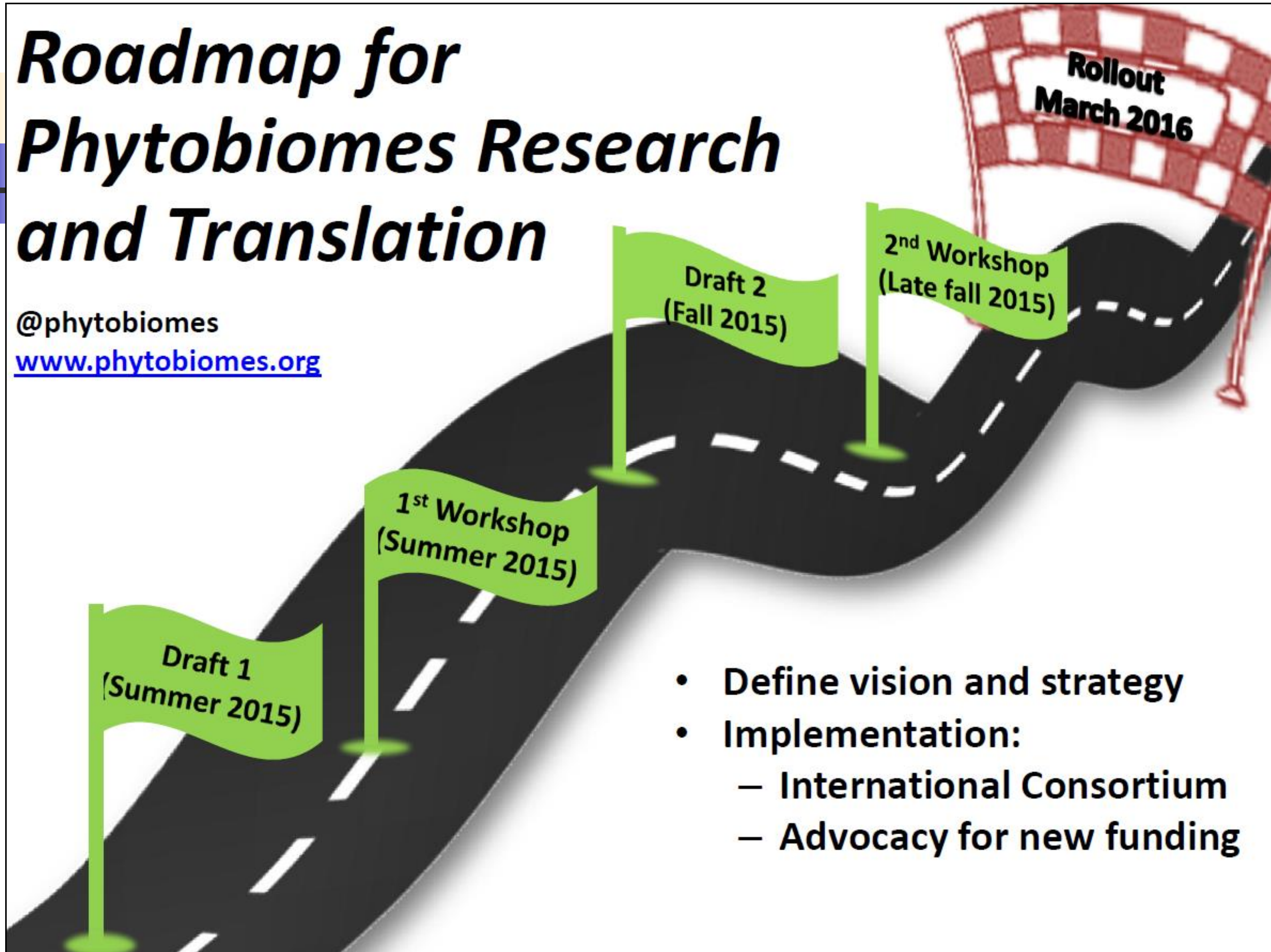


Source: UNEP

*1. 5 billion people depend on degraded lands for survival!

Jan E. Leach


Biota
Crop
Soil
Technology
Water
Data
Weather
Raj Khosla, Colorado State University



Visit www.Phytobiomes.org

Quorum sensing (QS)

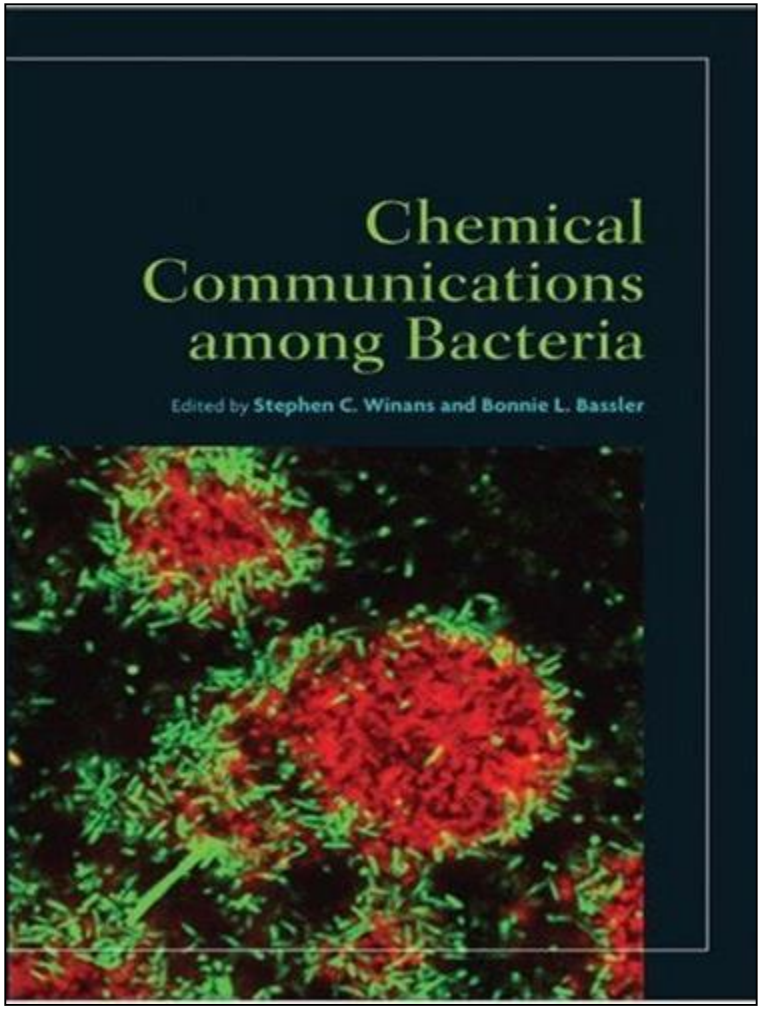
Autoinduction

Intra and inter-species molecular communications

Species-specific

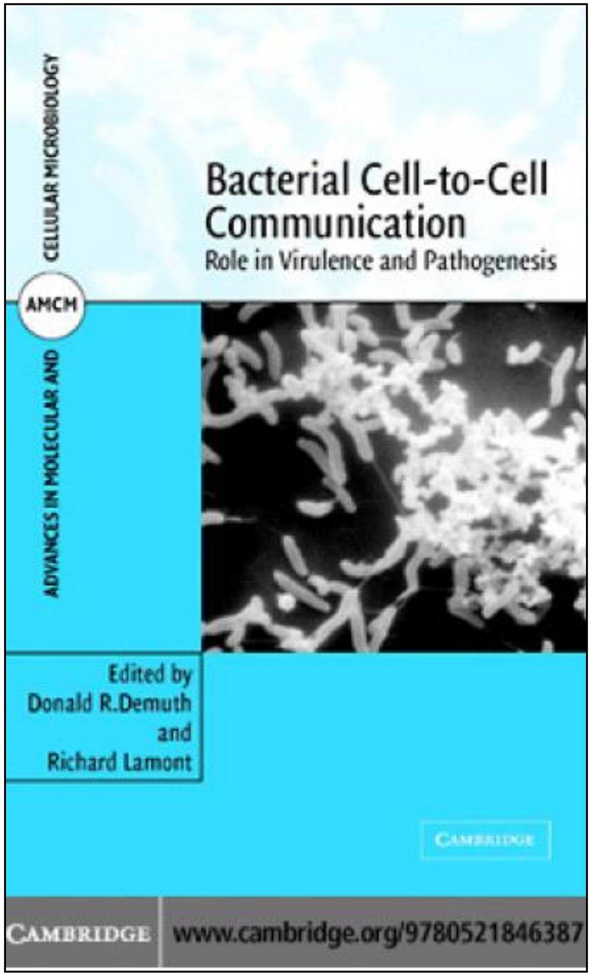
Chemical Communication among Bacteria

- **Chemical Communication among Bacteria**
- by Stephen C. Winans and Bonnie L. Bassler
- Hardcover: 483 pages
- Publisher: ASM Press; 1 edition (March 30, 2008).



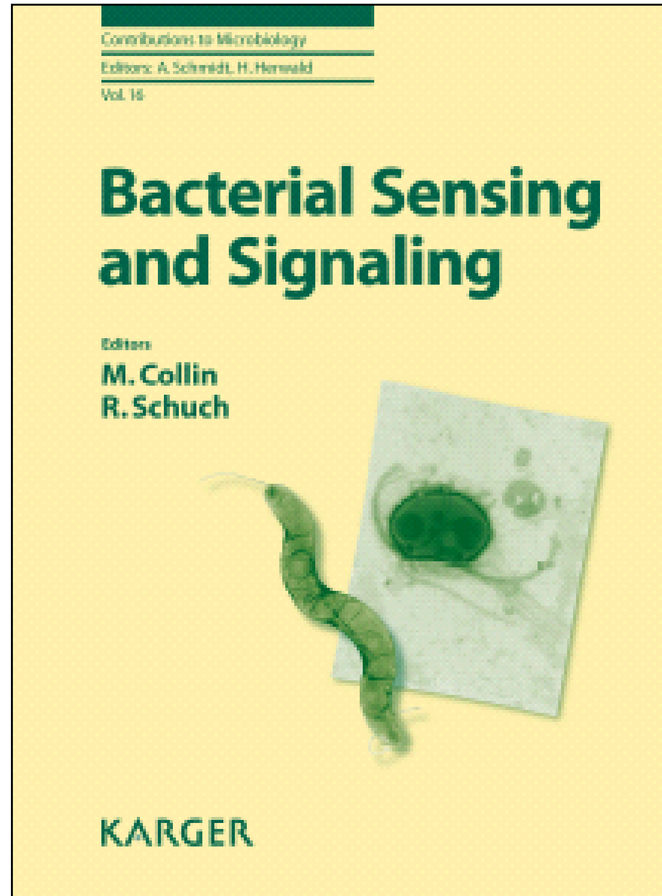
Bacterial Cell-to-Cell Communication
Role in Virulence and Pathogenesis

- **Bacterial Cell-to-Cell Communication- Role in Virulence and Pathogenesis**
- Donald R. Demuth and Richard J. Lamont
- Cambridge University Press 2006
- 338 pp.



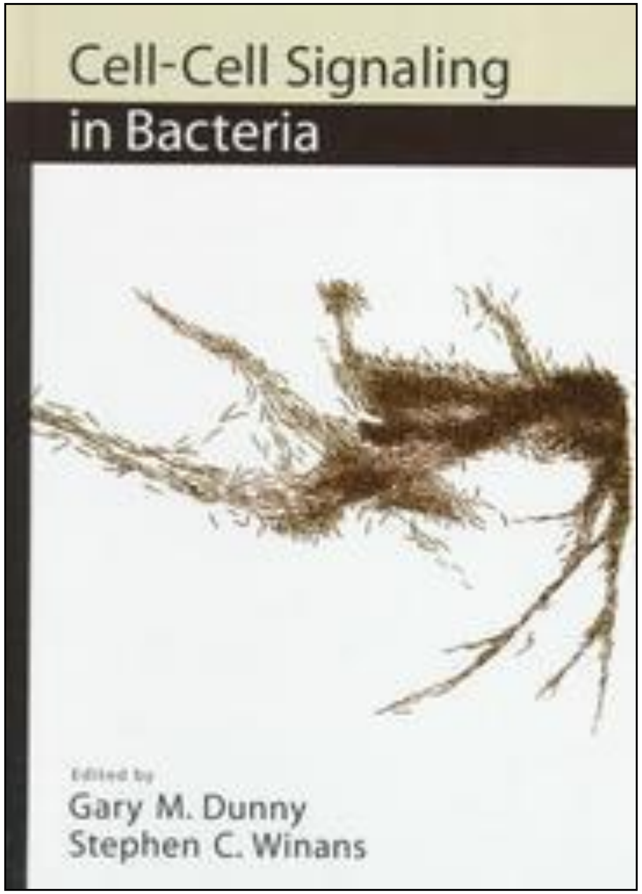
Bacterial Sensing and Signaling

- **Bacterial Sensing and Signaling**
- By Mattias Collin and Raymond Schuch
- Copyright 2009 by S. Karger AG (Switzerland)
- 230 pp.



Cell-Cell Signaling in Bacteria

- **Cell-Cell Signaling in Bacteria**
- by Gary M. Dunny and Stephen C. Winans
- Jun 1999
- Publisher: Amer. Society for Microbiology
- 367 pages.



The quorum sensing website

This web site is included in the ISI current web contents.

Main
Intro
Nott
Other
Links
Search

The University of Nottingham

PhD studentships and Post Doc position available

The quorum sensing site

The home of bacterial cell-cell communication on the web

The University of Nottingham Quorum Sensing Research Group

This site aims to be a "one stop shop" for quorum sensing research. It includes an overview of the field, links to researchers worldwide and descriptions of research carried out at Nottingham.

We now synthesize and supply a range of AHLs

Click here for details

Latest QS research papers

Bacterial Conversations: talking, listening and eavesdropping. A special issue dedicated to quorum sensing, issued by the Philosophical Transactions of the Royal Society can be read by following the link below:

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Last updated:
21/03/2011

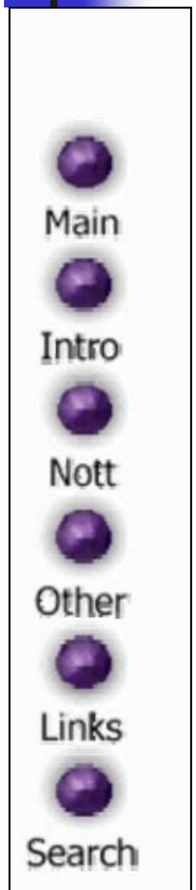
About the website

113894

Hits since Sept 2006

The quorum sensing website site

Research in other labs



- Europe
- South America
- Asia
- Africa
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- North America

The quorum sensing site

Research in other labs

In Asia

Main
Intro
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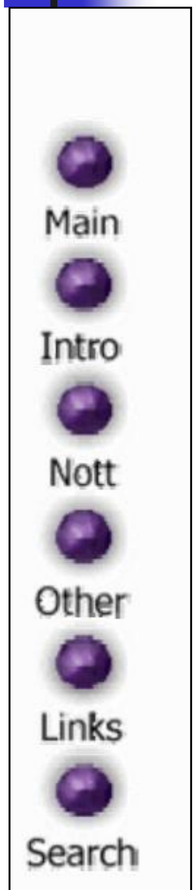
Links
Search

Singapore	Lian-hui Zhang The National University of Singapore	http://www.nus.edu.sg/uawards/2002/win8.htm
China	Baolin Sun University of Science and Technology	http://sunlab.ustc.edu.cn
India	Iqbal Ahmad Aligarh Muslim University	http://www.amu.ac.in/index.asp
India	Adline Princy SASTRA University	http://www.sastra.edu/scbt/scbt.html
India	Dipankar Chatterji Indian Institute Of Sciences	http://proteomics.mbu.iisc.ernet.in/dclab
India	S. Karutha Pandian Alagappa University	http://www.alagappabiotech.org/faculty/karutha%20pandian/index.htm
Iran	Saeed Tarighi Ferdowsi University of Mashhad	http://starighi.profcms.um.ac.ir/
South Korea	Chung Hak Lee Seoul National University	http://wemt.snu.ac.kr

The quorum sensing site

Links Categories

A list of synthesized AHLs



- **Links Categories**
- University of Nottingham
- Other universities
- Molecular biology
- Genome project
- Microbiology
- Scientific Societies
- Journals
- Search pages

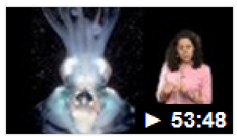
Below is a list of the AHLs which we synthesize and supply:
These chemicals can be purchased from Sigma-Aldrich-Fluka
To place an order please download and complete this form and email it to:
Darren Furniss (darren.furniss@nottingham.ac.uk) to whom you should address all other enquiries.

Bacterial quorum sensing

Videos of Bonnie Bassler talk on how bacteria communicate hosted by TED(Technology, Entertainment, Design)

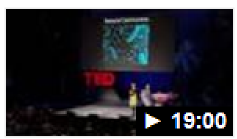
Bonnie Bassler: How bacteria "talk" | **Video** on TED.com
www.ted.com/.../**bonnie_bassler**_on_how_bacteria_communicate.ht...
TED Talks **Bonnie Bassler** discovered that bacteria "talk" to each other, using a chemical language **...** We have a fancy name for this: we call it **quorum sensing**.

Bonnie Bassler (Princeton) Part 1: Bacterial Communication via **...**



www.youtube.com/watch?v=saWSxLU0ME8
Mar 27, 2010 - Uploaded by ibioseminars
You need Adobe Flash Player to watch this **video**. **...** This process is called **quorum sensing** and it enables **...**

Bonnie Bassler: The secret, social lives of bacteria - YouTube



www.youtube.com/watch?v=TVfmUfr8VPA
Apr 8, 2009 - Uploaded by TEDtalksDirector
http://www.ted.com **Bonnie Bassler** discovered that bacteria "talk" to each other, using **...** You need Adobe **...**

Bonnie Bassler Part 1: Bacterial Communication via **Quorum ...**

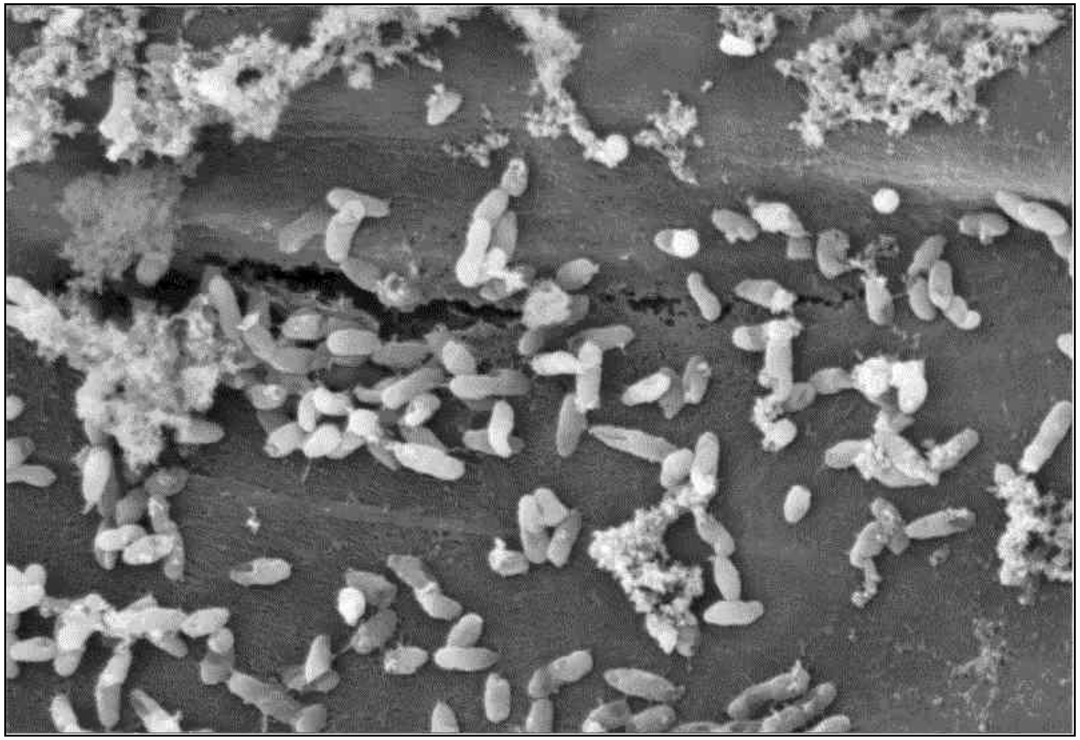
www.youtube.com/watch?v=qNkXDISo4ZI
Sep 10, 2011 - Uploaded by ibioseminars
Alert icon. You need Adobe Flash Player to watch this **video**. **....** **Bonnie Bassler** Part 2: Vibrio Cholerae **...**

Bonnie Bassler Discovers **Quorum Sensing** - YouTube

www.youtube.com/watch?v=1NoxOs-hcRU
Aug 10, 2008 - Uploaded by bigthink
Bonnie Bassler Discovers **Quorum Sensing**. bigthink·9,202 **videos**. Subscribe Subscribed Unsubscribe 301 **...**

Small Talk: Cell-to-Cell Communication in Bacteria

Bacteria make sense



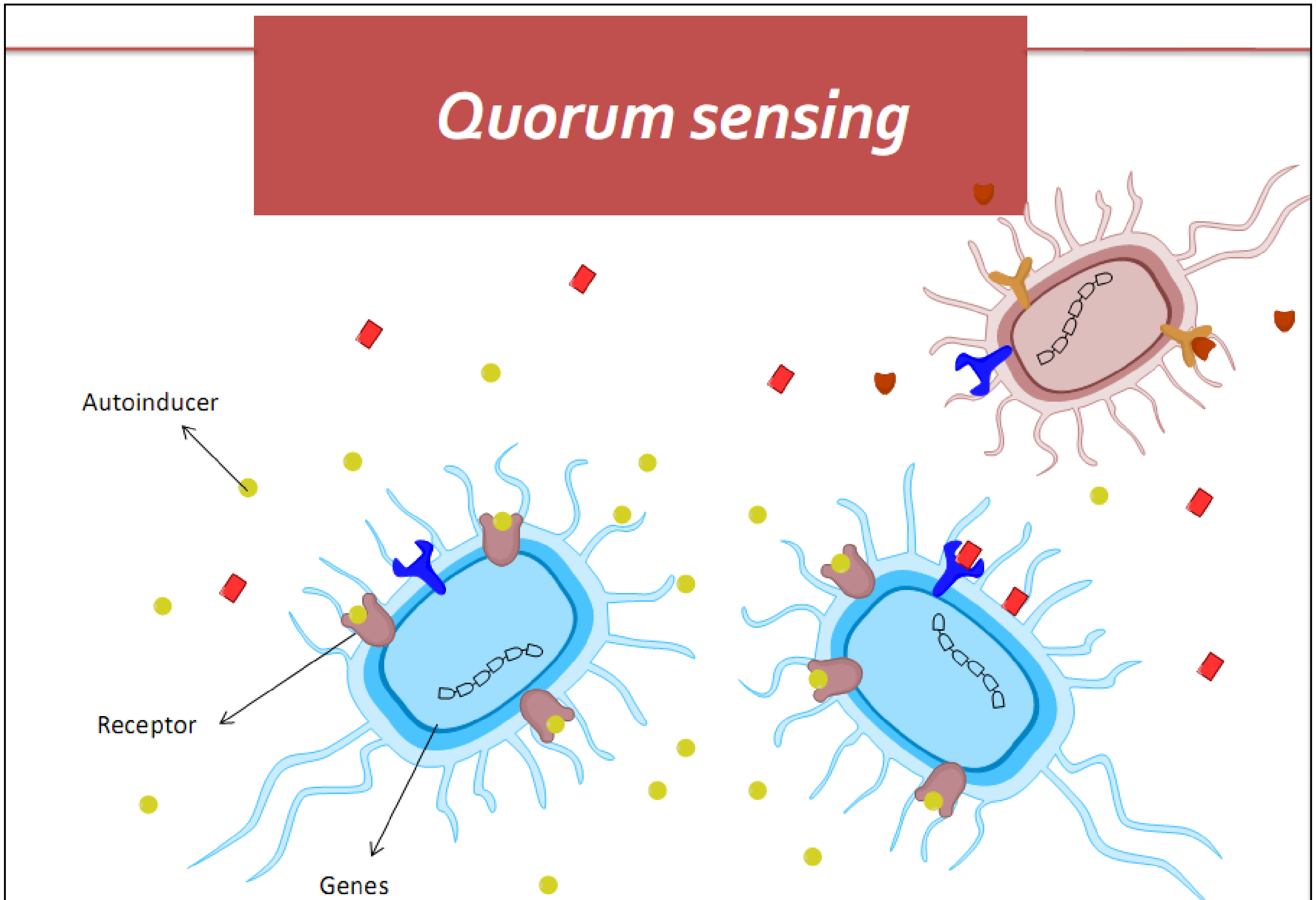
Research into AHL based quorum sensing started in the late 1960s (more than three decades ago).

Microbes seem to talk, listen and collaborate with one.

Bassler,2002; Hollowaym,2006

Small Talk: Cell-to-Cell Communication in Bacteria

Bacteria make sense



Camilo Gómez

Quorum sensing

The density-dependent cell-cell communication

- Bacteria live in colonies.
- Bacteria do not always act as single cells but have the ability to act as a population analogous to a multicellular organism.
- Quorum sensing" (QS) is the phenomenon which allows single bacterial cells to measure the concentration of bacterial signal molecules.
- QS enables bacteria to co-ordinate their behaviour.
- This form of signal dependent communication is present in:

1. Gram-negative
2. Gram-positive bacteria
3. Fungi.

Quorum sensing
Chemical communication signals
The fundamental biological process

- The autoinducer diffusible signal molecules mainly acyl-homoserine lactones(AHLs) are used by a diverse range of microorganisms such as:

1. Opportunistic pathogens such as *Pseudomonas* species;
2. Phytopathogens like *Erwinia* species;
3. Specific symbiotic strains as e.g. *Rhizobium* species;
4. Certain plankton phototrophic bacteria;
5. Signaling molecules can be sensed by fungi (*Candida albicans*; dimorphic; filamentous fungi);
6. AHLs also found in algal blooms.

Quorum sensing

Bacteria sophisticated communication systems

An important global gene regulatory mechanism

- QS is a beautiful invention because, by talking and listening to each other, the separate bacterial cells in a local population can co-operate with each other.
- QS coordinates:
- motility, stress responses, mating, nutrient acquisition, antibiotic production, exoenzyme secretion, and biofilm formation.
- QS mutants are defective in virulence and infectivity/stability.

Robinson *et al*.,2003;Chen *et al*.,2013

Quorum sensing
Factors affecting signalling process

- Thus, the signalling process is directly influenced by:

1. Abiotic factors (such as pH, temperature and medium composition), and
2. Biotic factors (such as other members of the bacterial community).

- These can modulate signal genesis, diffusion, interception and degradation and that can produce parasitic signals.

Boyer and Dye',2009

Quorum sensing
Bacterial communication systems
Autoinducer signals

- Bacterial communication systems include:

1. Long- and short-range chemical signaling channels;
2. One-way, two-way, and multi-way communication;
3. Contact mediated and contact-inhibited signaling; and
4. The use and spread of misinformation, or
5. More dramatically, even deadly information.

Bassler and Losick,2006

Quorum sensing

The background

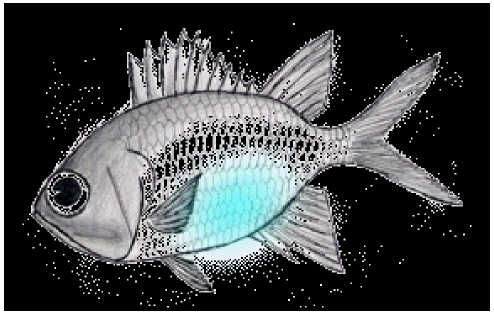
- The study of quorum sensing(bacterial signaling) has its roots in the late 1960s.
- Two scientists - J Woodland Hastings and Kenneth H Nealson discovered that a marine bacterium, *Vibrio fischeri*, produced light when its population reached a critical (late- log phase of growth).
- When fewer were present, the bacteria didn't bioluminesce.
- Therefore chemical communication first emerged from investigations into marine bacteria able to grow in the dark.

Quorum sensing
The background

- The two researchers speculated that the bacteria released a signal - something they called an autoinducer - that cried out:

  "We are here! We are here! We are here!"

- When the cacophony became loud enough, the assemblage glowed.
- In 1983, Michael R. Silverman, then at the Agouron Institute in La Jolla, California, and a colleague identified the genes for *V. fischeri*'s autoinducer and its receptor.



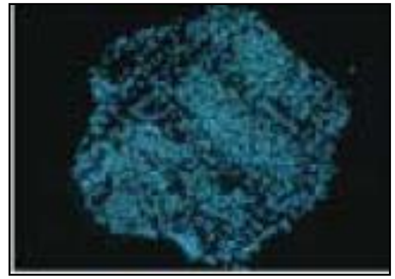
Bioluminescent bacteria

Bioluminescent bacteria and their role as light organs in the flashlight fish

- Pinecone fish utilize luminous bacteria, colonized in the ventral cavity, to:

1. Illuminate the surroundings as well as for
2. Intra-species communication.

Bioluminescent bacteria and their role as light organs in the flashlight fish.

	
(b) Colonies of *Photobacterium phosphoreum* photographed by their own light.	The flashlight fish *Photoblepharon palpebratus*; the bright area is the light organ containing bioluminescent bacteria.
	
Underwater photograph taken at night of *Photobacterium palpebratus* in coral reefs in the Gulf of Eilat.	Electron micrograph of a thin section through the light-emitting organ of *Photobacterium palpebratus*, showing the dense array of bioluminescent bacteria (arrows).

Diversity_1.pdf

Bioluminescent bacteria

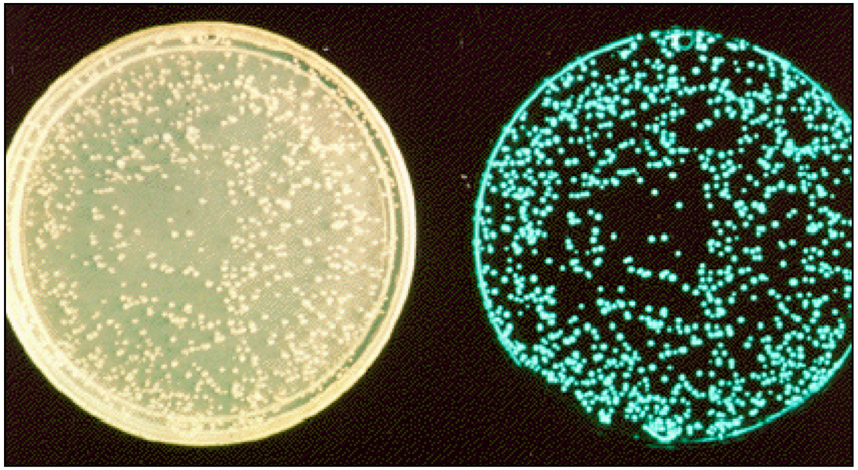
Making Seawater Complete (SWC) media

- Materials: natural or artificial seawater (you can get instant ocean salt mix from any pet store that sells marine fish), tap water, agar, yeast extract, peptone, glycerol, 20 sterile petri plates, container suitable to boil 1 liter of liquid, heat source to boil water SWC agar recipe:
- 1 L water
- 24 g sea salt
- 5 g peptone
- 3 g yeast extract
- 3 ml glycerol
- 15 g agar (if making solid media)

Bioluminescent bacteria

Bioluminescent bacteria and their role as light organs in the flashlight fish

- The picture at left shows how bioluminescent bacterial colonies appear on a petri plate under normal light.
- The image on the right is what you will see when you look at the same plate of bacteria in a dark room.



Light is only produced when the organisms are present at high cell densities.

Quorum sensing
Autoinducing signals

- QS has not only been described:

1. Between cells of the same species (intraspecies),
2. Between species (interspecies), and
3. Between bacteria and higher organisms (inter-kingdom).

Diggle *et al.*,2009

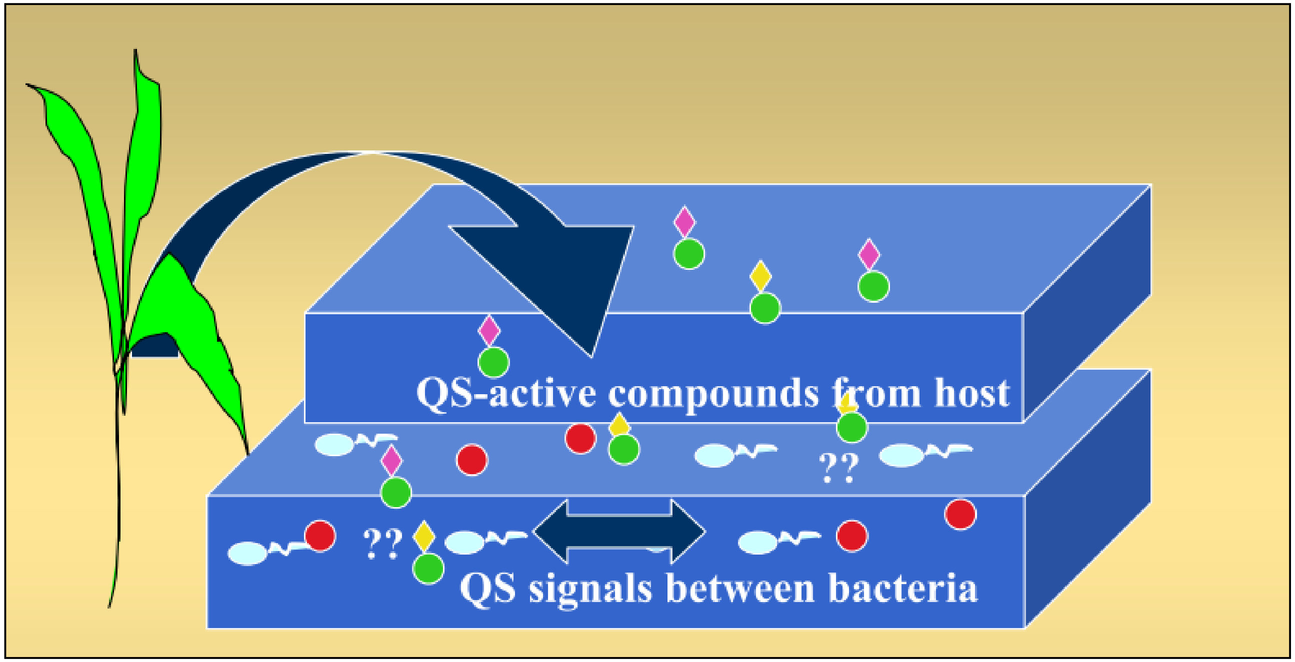
Quorum sensing
Bacteria-nematode communications
Interkingdom signaling

- *Caenorhabditis elegans* is a free-living terrestrial nematode that feeds on bacteria in its environment.
- The nematode *Caenorhabditis elegans* is also capable of adapting its behaviour in the presence of AHLs.
- Recent data show that *C. elegans* uses odors produced by bacteria to identify food sources.
- *C. elegans* could sense the acylated homoserine lactone (AHSL) autoinducers produced by many gram-negative bacteria possessing quorum-sensing (QS) systems.

Beale *et al*.,2006; Niu *et al*.,2010

Quorum sensing
Bacteria-Plant communications
Interkingdom signaling

- QS is crucial to many host-bacterial interactions.



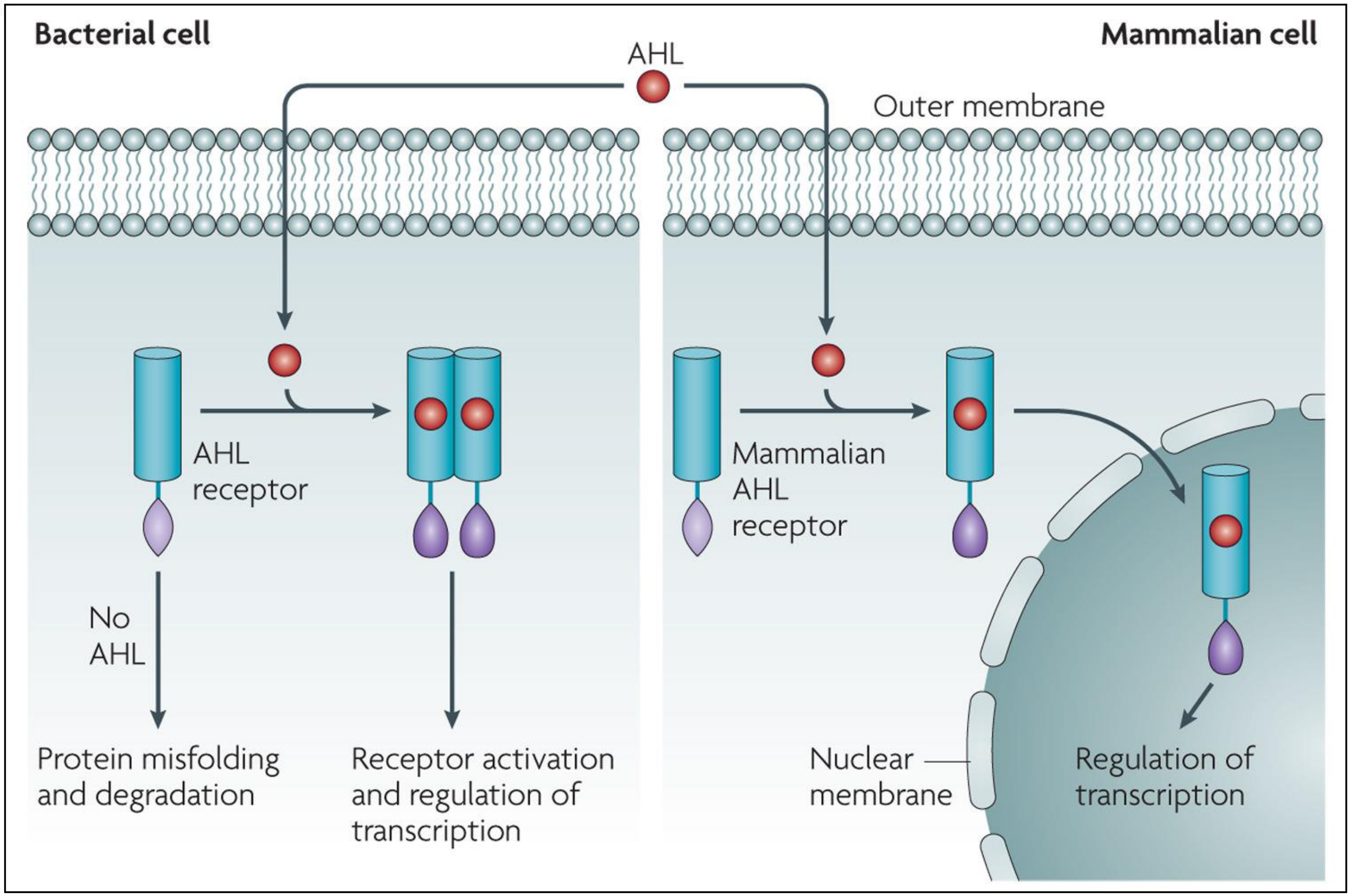
Robinson *et al.*,2003

Quorum sensing
Bacteria-Mammal communications
Interkingdom signaling

- In mammalian cells, AHLs also gain access to the cytoplasm by crossing the plasma membrane.
- The identity of the mammalian receptor (or receptors) for AHLs in mammalian cells is unknown.
- However, if there are intracellular receptors, it is proposed that the interaction with the AHL ligand activates these receptors and thereby allows their transportation into the nucleus, where they could control gene expression.

Hughes and Sperandio,2009

Quorum sensing
Bacteria-Mammal communications
Interkingdom signaling



Hughes and Sperandio,2009

Quorum sensing
Human pathogens

- **Quorum sensing** occurs in more than 70 types of microorganisms, including bioluminescent bacteria, which together produce light from a chemical reaction but alone must remain dark.
- Among human pathogens that use quorum sensing to estimate their population densities are:

1. *Pseudomonas aeruginosa*, which can cause serious infections, particularly in burn victims;
2. *Yersinia pestis*, the bubonic plague bacterium that killed millions during the Middle Ages.

Quorum sensing

The QS system

- The QS system can be seen as being based on the following crucial elements:

1. The autoinducers;
2. The signal synthase;
3. The signal receptor;
4. The signal response regulator;
5. The regulated genes (which form the so-called QS regulon).

A regulon is a collection of genes or operons under regulation by the same regulatory protein.

Nazzaro *et al.*,2013

AHL production
Gram negative bacteria

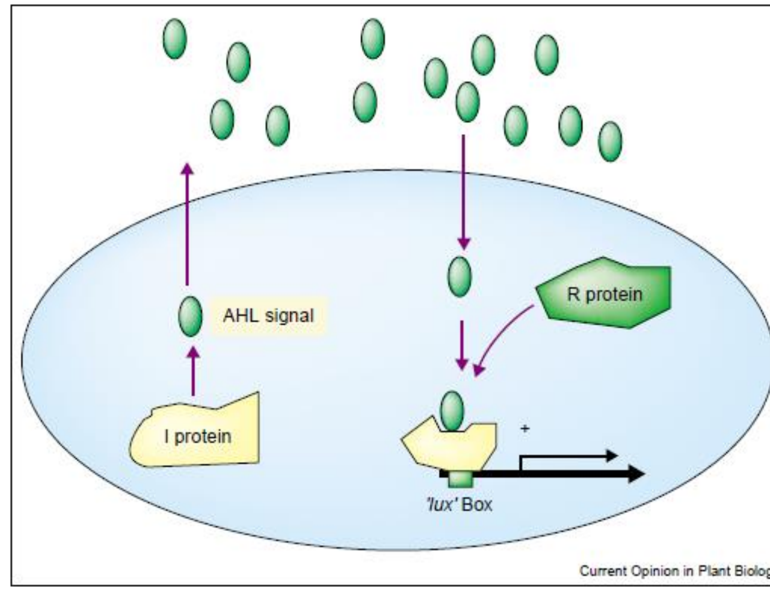
- *N*-acyl homoserine lactones (AHLs) are the best-studied QS signals among Gram-negative bacteria.
- Quorum sensing systems in Gram negative bacteria consist of two basic protein components:

1. I protein (synthesized by AHL synthases), and
2. R protein (AHL receptor).

- The concentration of the autoinducers (I proteins) are sensed and monitored by the response regulator family of transcription factors known as R proteins.

Quorum sensing
LuxI/LuxR system

- Bacterial cells (shown in blue) contain an I protein that is responsible for the synthesis of freely diffusible signals (green ovals).
- At high cell density, the signal accumulates intracellularly and interacts with the R protein.
- This interaction induces a conformational change in the R protein, which alters the affinity of the R protein for specific DNA sequences, known as '*lux*' boxes, that are located within the promoters of the AHL-regulated genes.

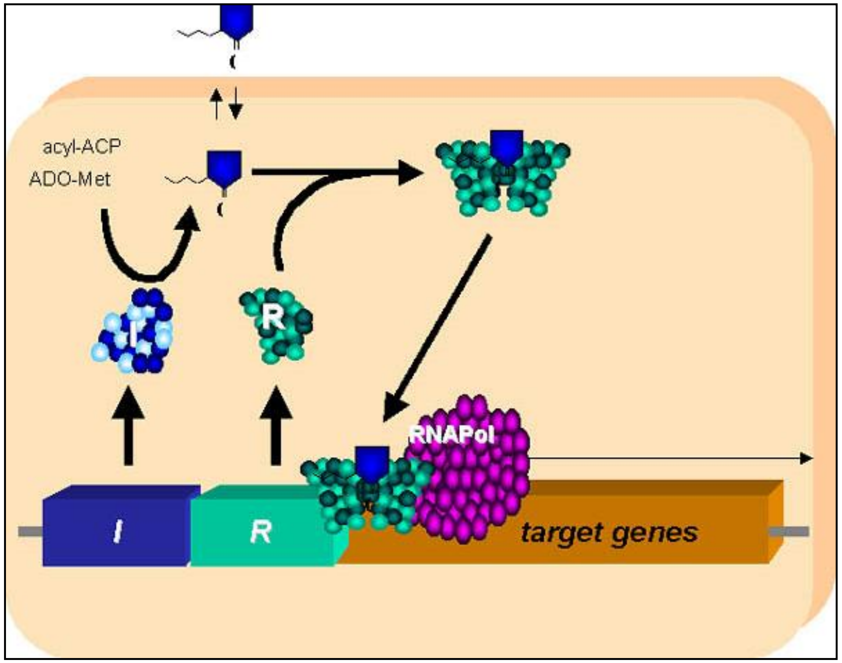


LuxI/LuxR is an integrated part of the lux operon, which is involved in bioluminescence through activation of the structural *lux* genes (lux*CDABE*).

Loh *et al.*,2002;..

A simplified model for AHL-mediated gene regulation in bacteria
In Gram negative bacteria

- The genes *I* and *R* represent the genes encoding the AHL synthase and regulatory protein, respectively.
- In the presence of sufficient AHL signal, the R regulatory protein is activated, possibly by dimerization.
- The activated R regulatory protein binds to a specific binding site and stimulates (or represses) transcription initiation by RNA Polymerase holoenzyme (RNAP).



RNA polymerase (RNAP) is the enzymatic machinery responsible for transcription, a key regulatory step in gene expression. The prokaryotic RNAP is a highly conserved, "crab claw" shaped enzyme with a molecular mass of ~400kD.

Pierson III,2005;..

A simplified model for AHL-mediated gene regulation in bacteria

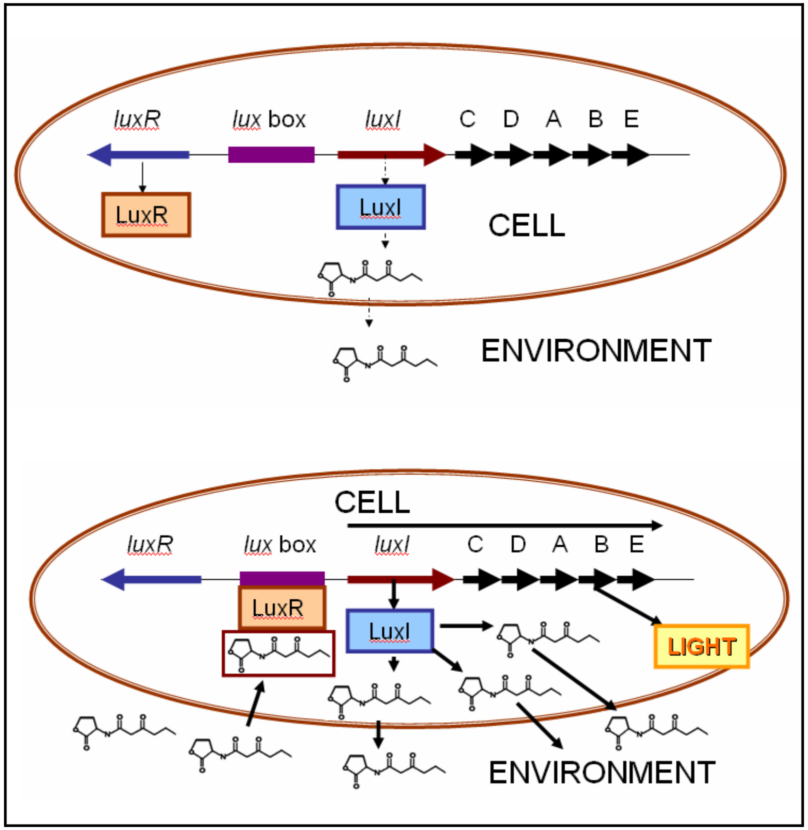
In Gram negative bacteria

Vibrio fischeri

a) When there are few bacteria nearby, the cell produces very little AHL.

b) As the concentration of cells in the surroundings increases, the signal accumulates.

- The AHL binds to LuxR and the LuxR/AHL complex binds to a region of DNA called the lux box.
- This activates the transcription of structural genes whose products give rise to luminescence.
- LuxI production also increases, leading to increased synthesis of the AHL.
- Homologues of the *V. fischeri* LuxR and LuxI proteins have been identified in over 25 species of gram-negative bacteria.

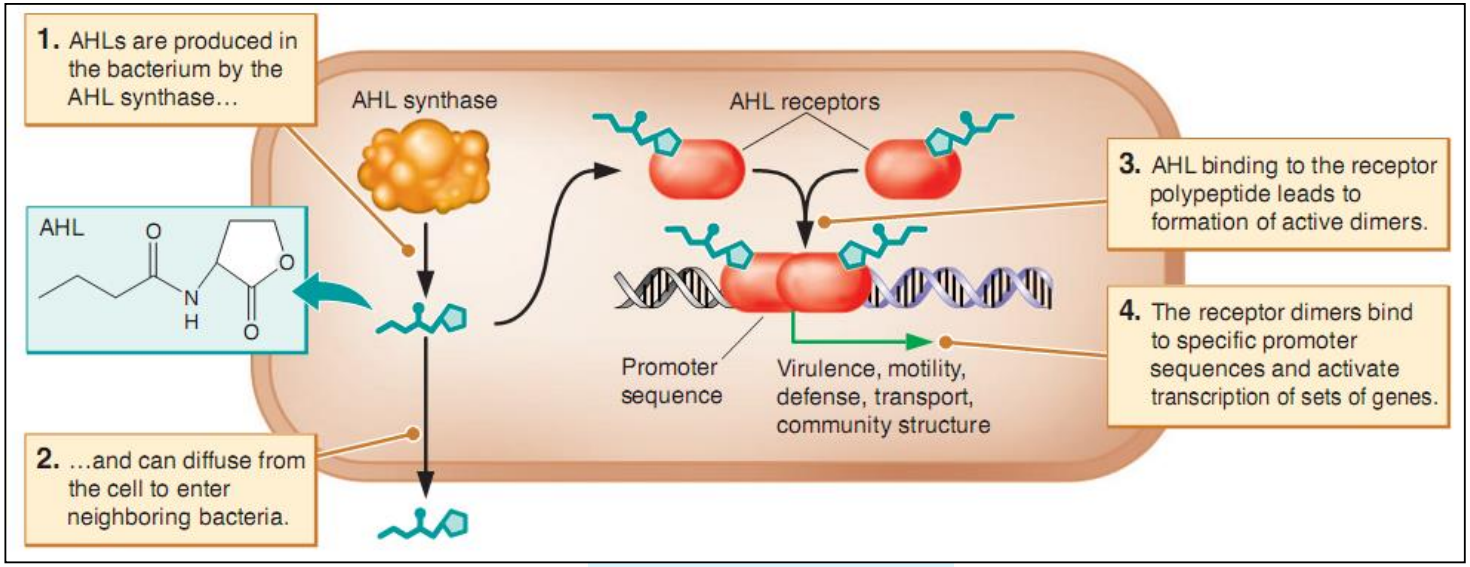


Lal,2009

Quorum sensing

Molecular mechanics of AHL-mediated QS

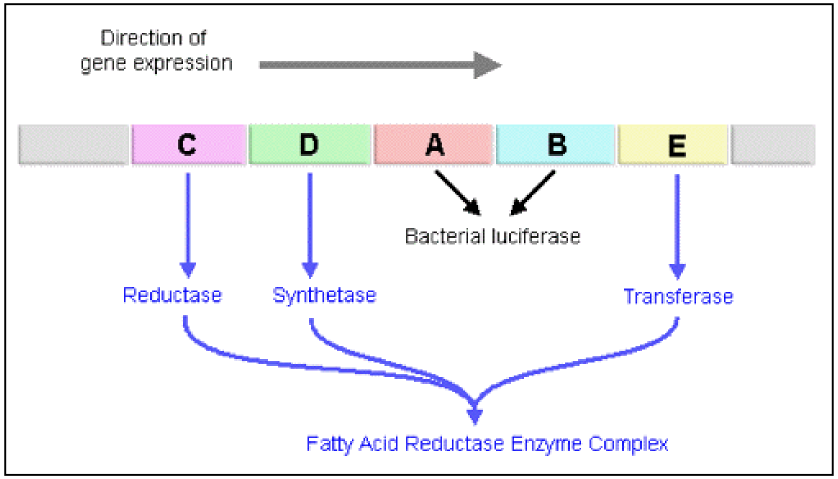
- AHLs are produced in the bacterium can diffuse from the cell to enter neighboring bacteria.
- AHL binding to the receptor polypeptide leads to formation of active dimers.
- The receptor dimers bind to specific promoter sequences in the bacterial genome and activate transcription of sets of genes.



Bauer *et al.*,2005

Quorum sensing
LuxR-type receptor
lux genes expression in mutant *E. coli*

- The constant light emission in luminous bacteria depends on several different enzymes which continuously generating the substrates for the bioluminescence reaction.
- In particular, the fatty acid reductase, a multienzyme complex, whose lux genes (*luxC, luxD*, and *luxE*) immediately flank the *lux*A and *lux*B genes of luciferase.



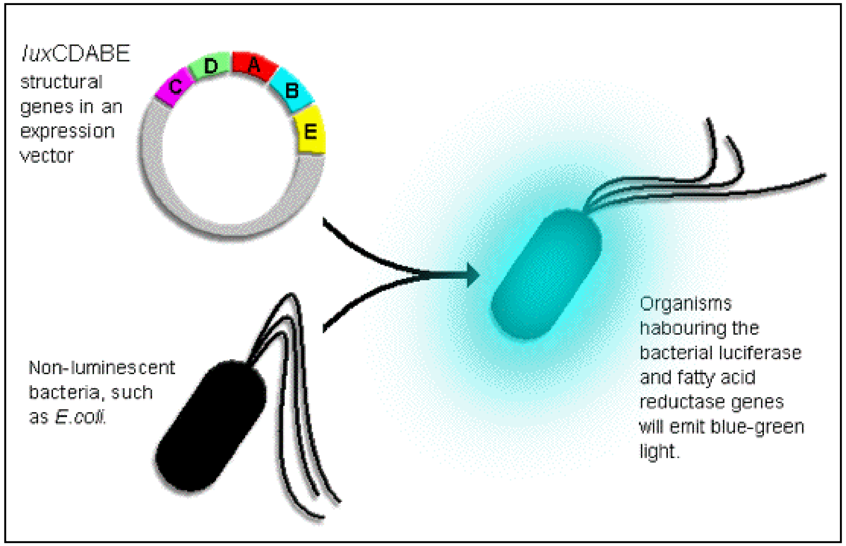
The arrangement of the *lux*CDABE open reading frames.

The light emission is due to transfer of electrons from a substrate, in presence of an enzyme called *Luciferase*. The electrons are transferred to a lower energetic level, with an output of energy in the form of light radiation.

Lin and Meighen 2009

Quorum sensing
LuxR-type receptor
lux genes expression in mutant *E. coli*

- The fact that the conversion of a nonluminous bacterium, such as *Escherichia coli*, to a light-emitter requires only the insertion of the *lux*CDABE genes, encoding the bacterial luciferase and the fatty acid reductase complex, into the cell.



Lin and Meighen,2009

Quorum sensing

Biomonitor/Bioreporter/Biosensor or reporter strains

1. *C. violaceum* Quorum-Sensing Circuits

- *Chromobacterium violaceum* CV026 synthesizes and responds to C6-HSL.
- The AHLs induce the production of a purple compound violacein which is visualized on agar plate.
- It is more sensitive to C4 to C8 un-substituted homoserine lactones.
- Consistent with the inability of long chain AHL molecules to induce CviR transcriptional activation, C10-HSL, C12-HSL, and C14-HSL have been reported to antagonize the CviR protein.

Swem *et al.*,2010;…

Quorum sensing

Biomonitor/Bioreporter/Biosensor or reporter strains

Chromobacterium violaceum CV026

Strain/Plasmid	Host	Based on QS system	Reporter system	Best responds to	Good detection	Commonly used for
C. violaceum CV026	*C. violaceum*	CviI/R (*C. violaceum*)	Violacein pigment	C6-AHL	C6-3-oxo-AHL C8-AHL C8-3-oxo-AHL C4-AHL	T.S., TLC

*T.S. refers to 'T' streak analysis in solid media.
Note that TLC analysis of unusually long AHLs results in no migration.

Steindler and Venturi,2007

Biomonitor/Bioreporter/Biosensor or reporter strains
Broad-spectrum AHL TraR-dependent *A. tumefaciens* NTL4 (pZLR4) biosensor

2. ***Agrobacterium tumefaciens* NTL4 (pZLR4) ATCC® BAA-2240™ :** *A. tumefaciens* strain NTL4 containing plasmid pZLR4 that carries a *traG::lacZ* reporter fusion and *traR*.

- In the presence of AHLs with long acyl chains the TraR protein is activated, transcription of the *traG::lacZ* fusion is turned on, and LacZ (β-galactosidase) activity can be used as a reporter of *traG* transcription.
- *A. tumefaciens* reporter strain NT1 detects a broad range of AHSLs (good sensitivity on TLC) and was used for the detection of both short (C6) and long acyl chains (C12) AHL suppression.
- Anti-QS compounds inhibit the QS-dependent promoter and subsequent *lacZ* expression, thus limiting X-gal hydrolysis, and the appearance of blue color.

Adonizio,2008; González *et al*.,2009; Koh *et al*.,2013

Methods

Biomonitor/Bioreporter/Biosensor or reporter strains

Agrobacterium tumefaciens NTL4 (pZLR4)

Strain/Plasmid	Host	Based on QS system	Reporter system	Best responds to	Good detection	Commonly used for
pZLR4	*A. tumefaciens* NT1	*tume*TraI/R (*A. faciens*)	β-galactosidase	C8-3-oxo-AHL	All 3-oxo-AHLs C6-AHL C8-AHL C10-AHL C12-AHL C14-AHL C6-3-hydroxy-AHL C8-3-hydroxy-AHL C10-3-hydroxy-AHL	T.S., TLC, Q.

T.S. refers to 'T' streak analysis in solid media.
Note that TLC analysis of unusually long AHLs results in no migration.
Q refers to quantification.
Quorum sensing uses a low molecular weight chemical signal (almost all belonging to the chemical family called N-oxoacylhomoserine lactones (Szenthe and Page,2003). The main 3-oxo-AHLs are: C6-3-oxo-AHL, C8-3-oxo-AHL; C10-3-oxo-AHL, C12-3-oxo-AHL, C14-3-oxo-AHL, C16:1-3-oxo-AHL.

A. tumefaciens NT1 (or NTL4) is a broad-spectrum AHL TraR-dependent biosensor. HSLs with acyl-chain length of C6 to C12 are readily detected by the indicator strain. NTL4 (pZLR4) responds preferentially to 3-oxo-C8-HSL and to unsubstituted and 3-oxo signals, except for C4-HSL.

Steindler and Venturi,2007;..

Methods

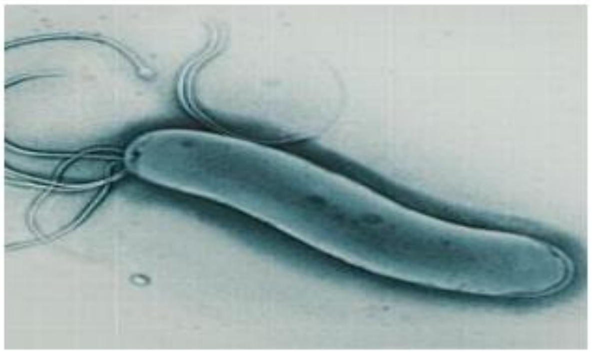
Biomonitor/Bioreporter/Biosensor or reporter strains

3. ***P. aeruginosa*** **PAO1(ATCC 27853):** A wild type strain of *P. aeruginosa* which is used for anti-swarming bioassays.
4. ***Escherichia coli*** **(pSB401);**
5. ***E. coli*** **(pSB1075);**
6. ***P. putida*** **F117 (pAS-C8);**
7. ***Serratia liquefaciens*** **MG44 (pJBA132).**

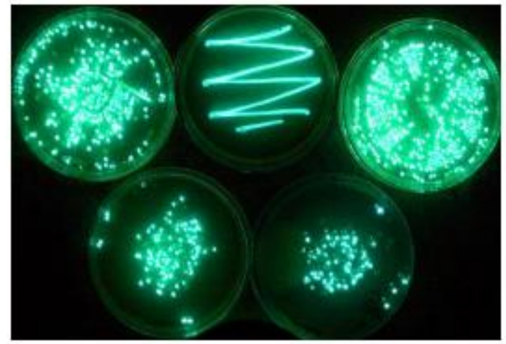
Please note that different biosensor strains of *E. coli* respond to a range of different AHLs. E.g.
-*E. coli* (pSB401) produces bioluminescence in the presence of short chain AHL.
-The strain *E. coli* (pSB403) is able to respond to a range of different AHLs by luciferase production (e.g., BHL, HHL, OHHL, and ODHL).
-Biosensors *E. coli* JM109 [pSB1075] and *E. coli* JM109 [pSB536] for detection of Long (C12) and short chain AHLs (C4), respectively.

Steidle *et al.*,2001;Adonizio,2008; Koh *et al.*,2013;..

Quorum sensing
Some other functions

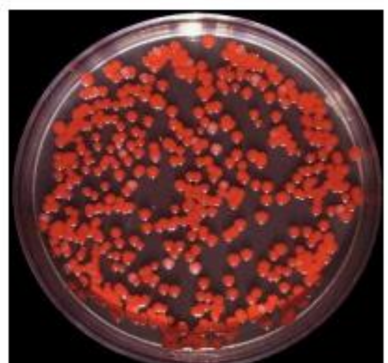


Virulence

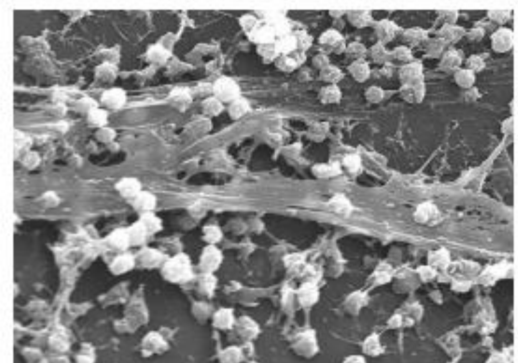


Bioluminiscence

Swarming



Pigments



Biofilms

Quorum sensing
Synthesis of Acyl-HSLs

- Gram-negative LuxIR systems:
- This system exhibits great specificity.
- The autoinducer (AI/AHL)molecule produced by one species of bacteria can rarely, if ever, interact with the LuxR-type regulator of another species.

Kaper and Sperandio,2005

Quorum sensing

Autoinducing peptide (AIP) QS in Gram-positive bacteria

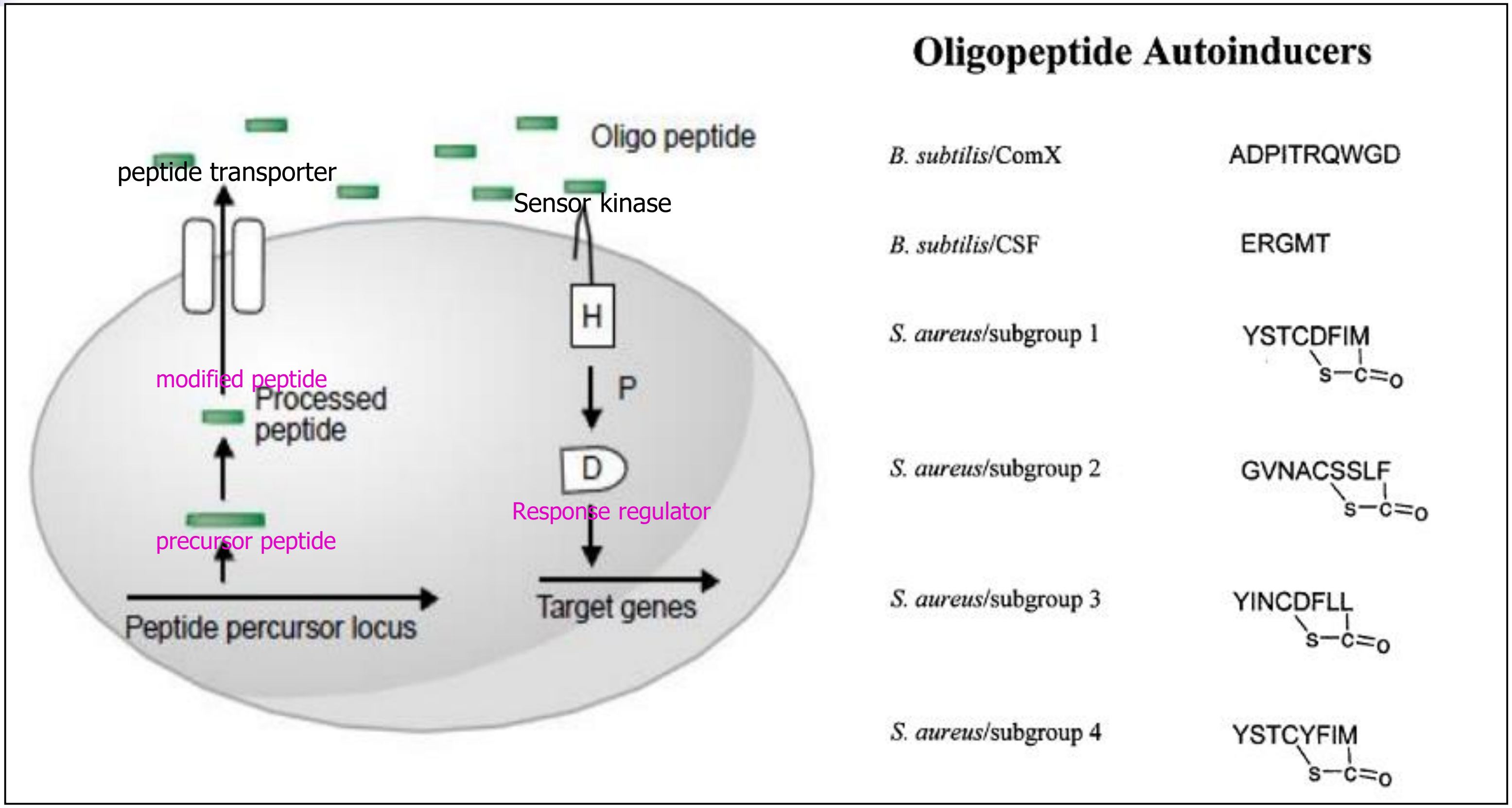
- QS systems in Gram-positive bacteria are much more diverse than the Gram-negative AHL QS system and there are many variations in the nature of the QS signal.
- Gram-positive bacteria speak with oligopeptides.
- The QS system of Gram-positive bacteria generally consists of:

1. A signal peptide, and
2. A two-component regulatory system made up of a membrane-bound sensor, and
3. An intracellular response regulator.

Note: The receptors for AHLs in G-ve bacteria exist in the cytoplasm, whereas in the case of G+ve bacteria these are located on membrane (membrane receptor).

Autoinducing peptide (AIP) QS in Gram-positive bacteria

In Gram positive bacteria



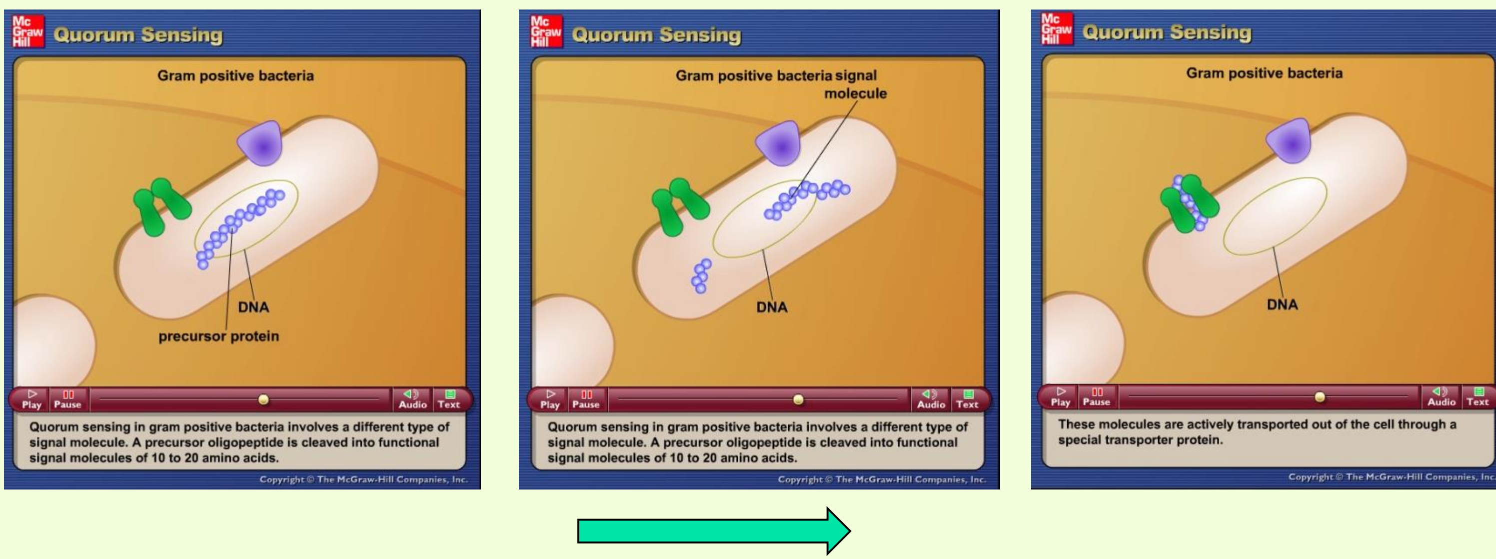
Oligopeptide signalling molecules have only been found in Gram positive bacteria.
The precursor peptide autoinducers are modified and transported.

Camilo Gómez

Autoinducing peptide (AIP) QS in Gram-positive bacteria

Autoinducing peptides (AIPs)

- The precursor peptide autoinducers are modified and transported out of the cell by ATP-binding cassette exporter complex.

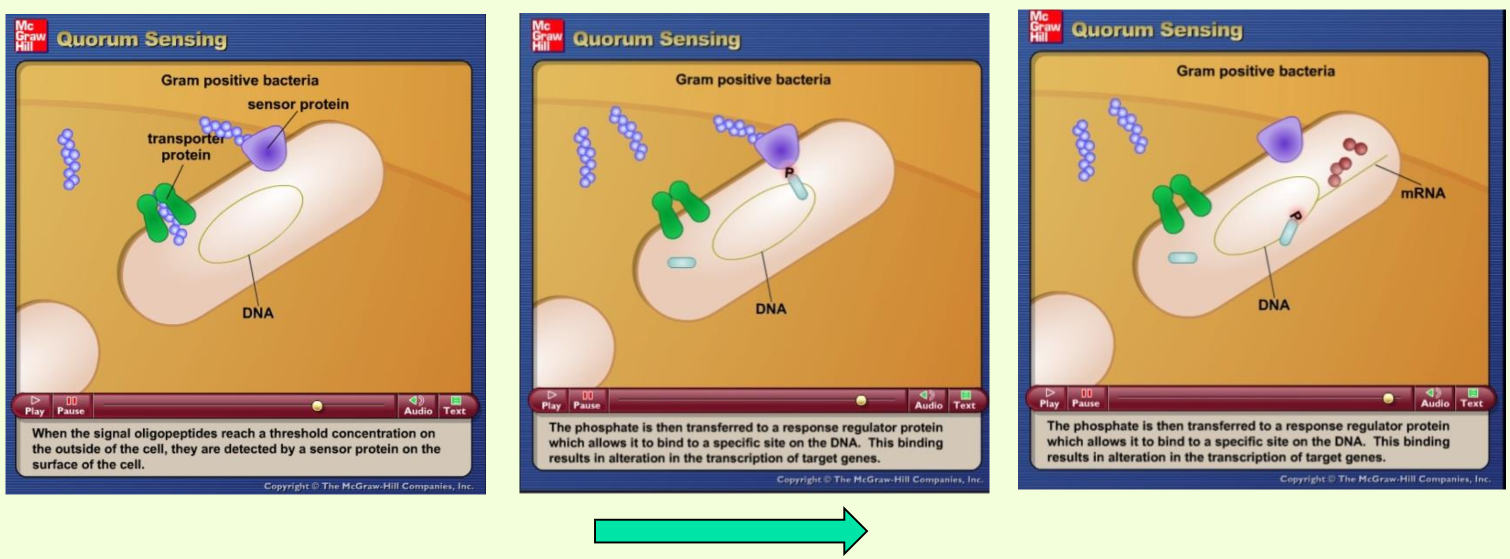


Rutherford and Bassler,2012;Koh *et al.*,2013

Autoinducing peptide (AIP) QS in Gram-positive bacteria

Autoinducing peptides (AIPs)

- The oligopeptide binds to a cognate membrane-bound two-component histidine kinase receptor.
- The phosphorylated response regulator activates transcription of the genes in the QS regulon.

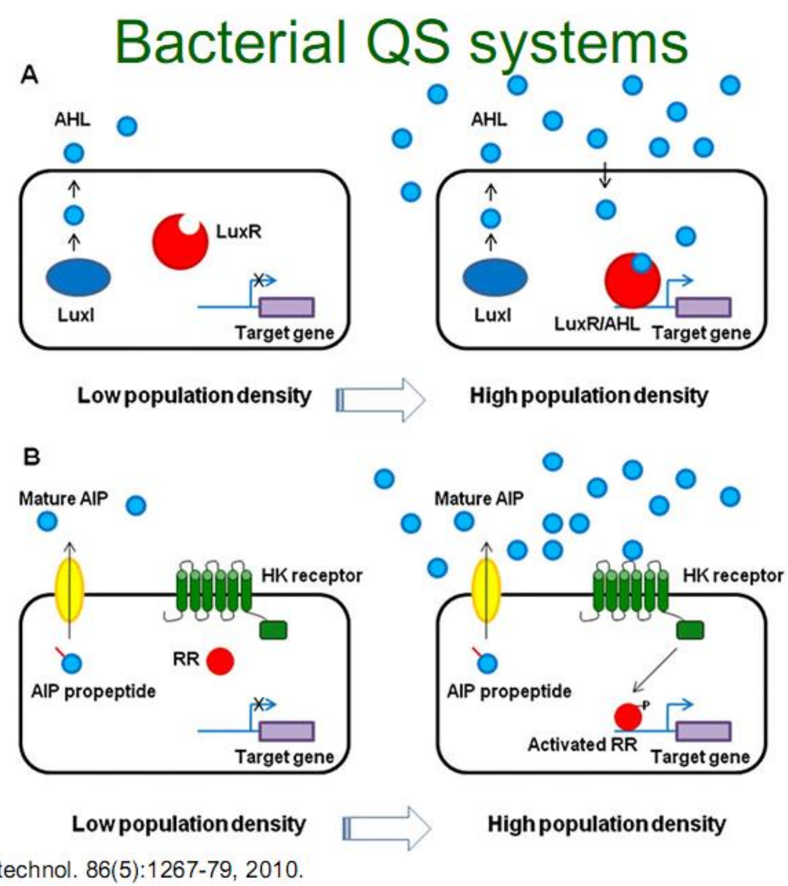


Rutherford and Bassler,2012;Koh *et al.*,2013

Bacterial Quorum Sensing
Gram-Positive vs. Gram-negative bacteria

AHL in gram-ve bacteria

Autoinducing peptides (AIPs) in gram+ve bacteria



Haung,2011

Bacterial Quorum Sensing

Gram-Positive vs. Gram-negative bacteria

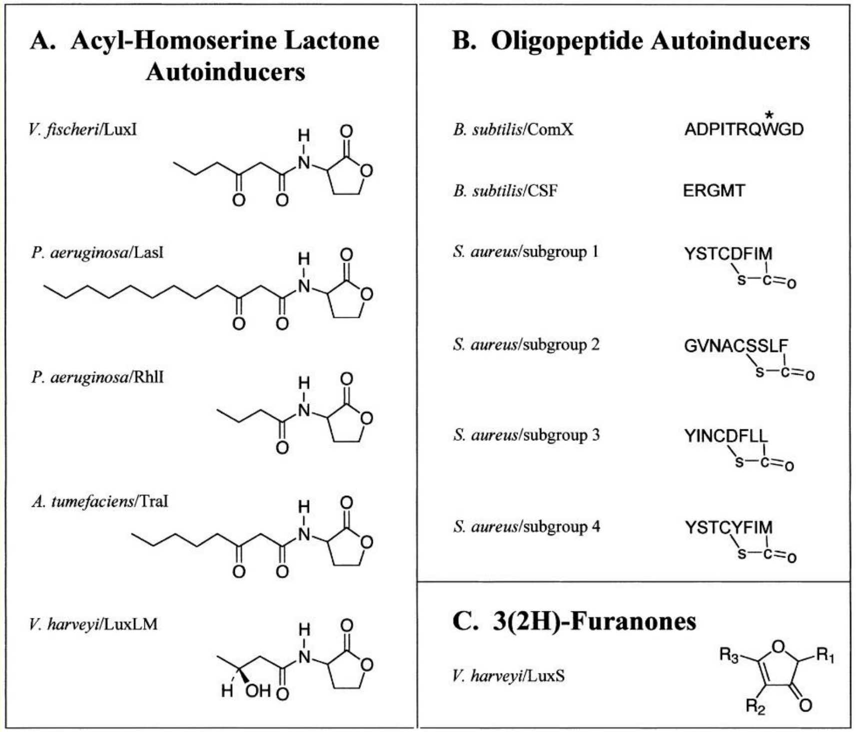
- Three classes of molecules used as autoinducers:

A. Representative AHLs used by Gram-negative bacteria.

B. Representative oligopeptide autoinducers used by Gram-positive bacteria.

C. The general structure of the 3(2H) class of furanones. A 3(2H)-furanone is suspected to be the LuxS-dependent autoinducer called AI-2.

- The latter found both in Gram-ve and Gram+ve bacteria.

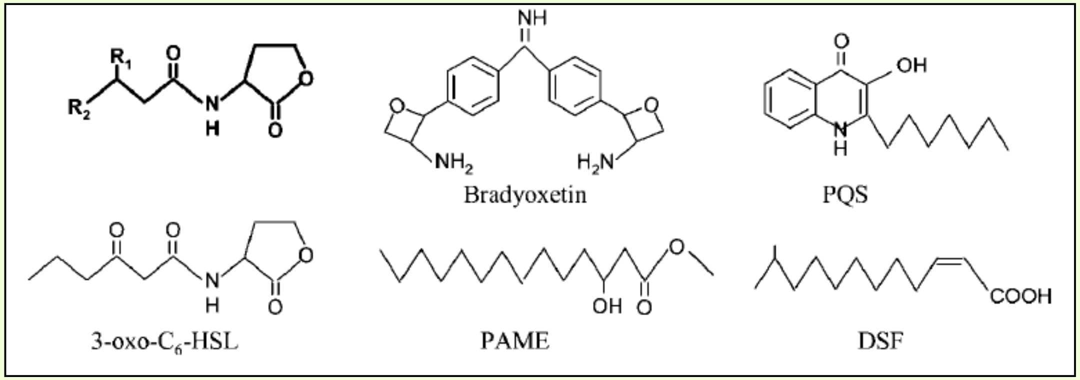


Schauder and Bassler 2001;..

Structures of AHL signals and non-AHL autoinducers

Gram negative bacteria

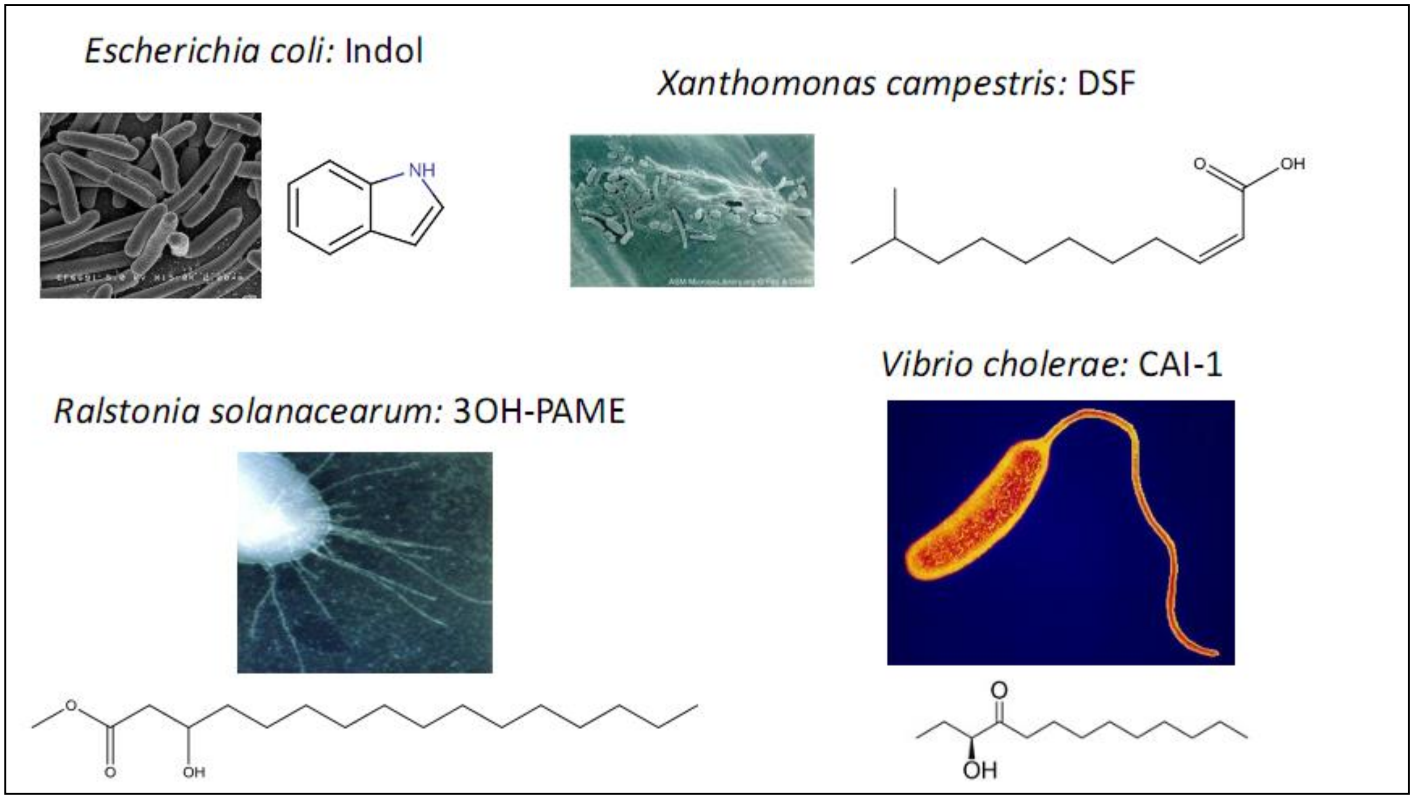
- **AHL signals:**
- N-Acylhomoserine lactones (AHLs).
- 3-oxohexanoylhomoserine lactone (3-oxo-C6-HSL) produced by several plant pathogens.
- **Non-AHL signals:**
- Bradyoxetin identified in *Bradyrhizobium japonicum*.
- 3-OH PAME (3-hydroxypalmitic acid methyl ester) produced by *Ralstonia solanacearum*.
- DSF (diffusible signal factor, cis-11-methyl-2-dodecenoic acid) of *Xanthomonas campestris*.



Soto *et al.*,2006

Structures of AHL signals and non-AHL autoinducers

Gram negative bacteria



Microbiol Rev 2008, 32(5):842-57.

Camilo Gómez

Structure & Functions of AHLs

Some diffusible signals utilized by Gram +ve and-ve bacteria

Signal	Mediates
N-acyl-homoserine lactones (AHLs)	Antibiotic synthesis, virulence gene expression, etc.
Butyrylactones	Antibiotic synthesis in *Streptomyces* spp.
Amino acids	Swarming in *Proteus* spp.
Peptides	• competence in *Bacillus* spp. • fruiting body formation in *Myxococcus* spp. • conjugal plasmid transfer in *Enterococcus* spp.

In Gram-positive bacteria, such as *B. subtilis* oligopeptides are the signalling molecules (Lazdunski *et al*.,2004)

Pierson III,2005

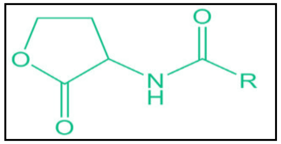
Autoinducers

Gram-negative bacteria

Six types of autoinducers

- Two main types of AIs have been described in Gram-negative bacteria (AI-1 and AI-2).

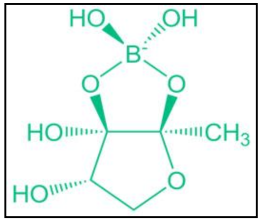
1. **AI-1 molecules or Acyl homoserine lactones (AHLs). Also called autoinducer 1(AI-1):**



- Used for intraspecies communication.
- Example: in *Vibrio fischeri* and many other Gram-negative bacteria such as *Erwinia* spp., and *Escherichia* spp.

2. **AI-2 is a unique furanosyl borate diester. Also called Autoinducer 2(AI-2) or LuxS:**

- It is considered to be a universal system used for interspecies communication (between species).
- Example: in *Vibrio fischeri* and *Salmonella enterica.*
- Exception: it is also found in Gram+ve bacteria.



Autoinducers

Gram-negative bacteria

Five types of autoinducers

3. **AI-3. Also termed as Autoinducer 3 (AI-3)**

- A new bacterial signal was decoded in some species of Gram-negative bacteria which are resident in the human gastrointestinal tract and might also be involved in inter-kingdom signaling.

4. **Cyclic dipeptides:**

- A new class of autoinducers was recently identified in strains of *Pseudomonas*.

5. **Bradyoxetin:**

- A cell density factor was proposed to be involved in population density-dependent regulation of the *nod* genes in *Bradyrhizobium japonicum*.

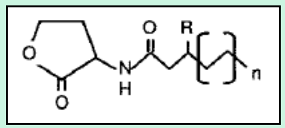
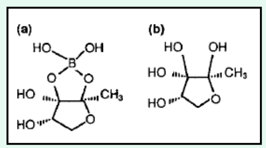
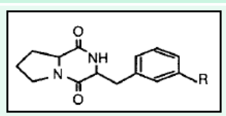
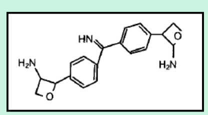
6. **Diffusible signal factor (DSF):**

- In *Xanthomonas* spp. and few more Gram-ve bacteria.

Autoinducers

Gram-negative bacteria

Five types of autoinducers

Autoinducer(s)	General structure	Producing species	Phenotype(s) regulated
AHL(AI-1)		Many Gram-ve bacteria	Motility, exopolysaccharides, biofilms, others
AI-2 (LuxS)		Both Gram-ve and Gram+ve bacteria	Bioluminescence, ABC transporters
AI-3		Some species of Gram-negative bacteria	responsible for activating gene expression in *Salmonella*
Cyclic dipeptides		*P. fluorescens, P. alkaligenes*	Cross activate quorum sensing indicator strains
Bradyoxetin		*B. japonicum*	Nodulation
DSF		*X. campestris*	Endoglucanase production

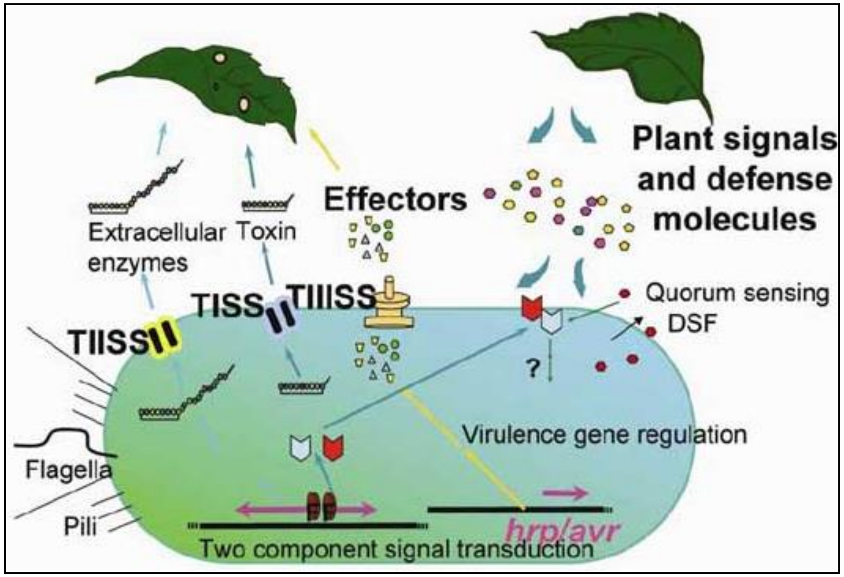
González and Keshavan,2006;..

AHL signals in Gram-ve bacteria

Non-acyl HSL-based systems

Diffusible signal factor (DSF and BDSF)

- DSF originally identified from the plant bacterial pathogen *Xanthomonas campestris* pv. *campestris*.
- BDSF was found from *Burkholderia cenocepacia*.
- In these cases, two QS systems appear to act in conjunction in the regulation of *B. cenocepacia* virulence (fingertip rot disease of banana).



Three secretion systems:
TISS,TIISS and TIIISS

Chatterjee,2009; Deng *et al.*,2010; Wu *et al.*,2011

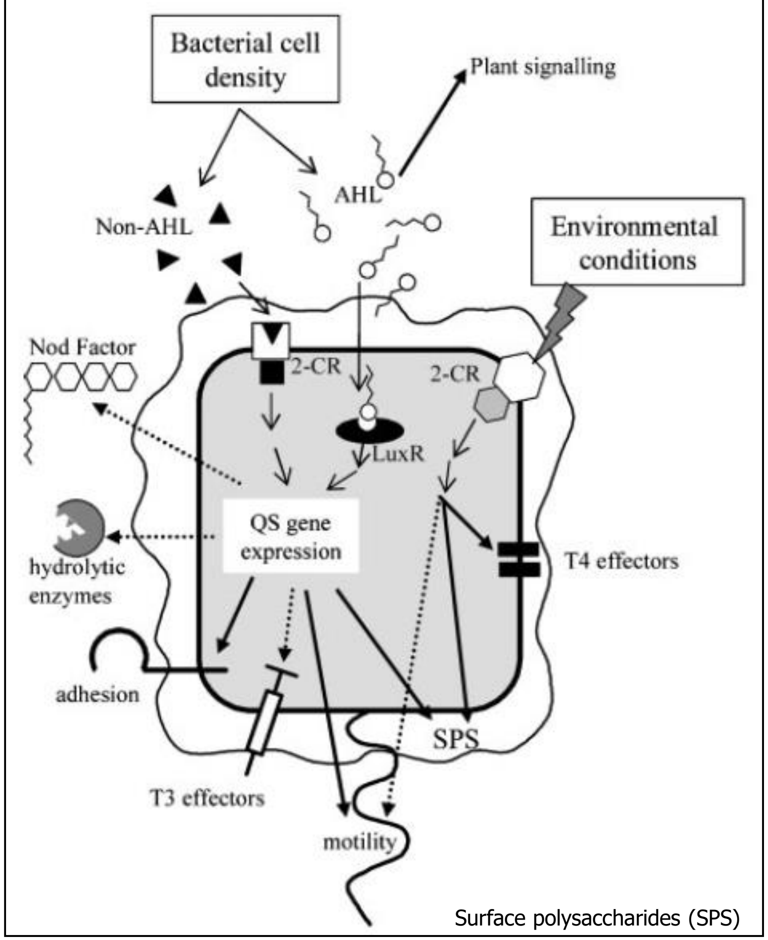
DSFs: non-AHL signals

DSF-based quorum sensing

Xanthomonas spp.

- Host colonization and invasion mediated by quorum sensing (QS) signals and two component regulatory (2-CR) systems.

1. Detection of N-acylhomoserine lactones (AHL, loop and tail) by cytoplasmic LuxR-type transcriptional activators (black oval), and
2. Non-AHL (black triangles) by 2-CR systems (white and black squares), allow plant interacting bacteria to coordinate the expression of important genes for host colonization and invasion in response to cell density.



Surface polysaccharides (SPS)

Soto *et al.*,2006

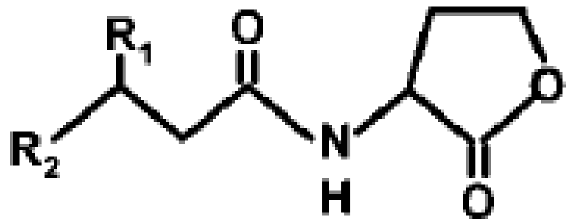
AHL signals in Gram-ve bacteria
Structure of acyl-HSLs

- AHL was the very first QS signal to be detectedin Gram-negative bacteria (Eberhard *et al*.,1981).
- It consists of a lactonized homoserine moiety attached by an amide bond to an acyl side chain. Homoserine lactone ring is conserved and connected to a variable acyl chain.
- The acyl side chain, however, can vary in:

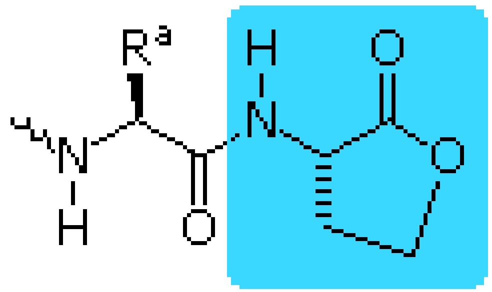
1. Length, and
2. Degree of substitution.

AHL autoinducers all share a common homoserine lactone moiety and differ only in their acyl group.

Fine structure of N-Acylhomoserine lactones (AHLs)



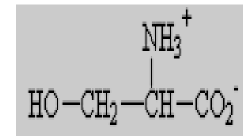
Acyl homoserine lactone (AHL)



Lacton moiety of AHL

Acyl homoserine lactones are lactone rings with a carbonyl tail (C_4-C_{16}) with varying functional lengths (Nagy,2010).
Lactones are cyclic esters, a ring of two or more carbon atoms and a single oxygen atom with a ketone group at one of the carbons adjacent to the other oxygen.

Fine structure of N-Acylhomoserine lactones (AHLs)



L- serine(ser)

- L-Homoserine is not one of the common amino acids encoded by DNA.
- Homoserine (also called isothreonine) is an α-amino acid with the chemical forumula: $HO_2CCH(NH_2)CH_2CH_2OH$.
- L-Homoserine differs from the amino acid serine by insertion of an additional -CH2-unit into the backbone.
- Homoserine, or its lactone form, is the product of a cyanogen bromide cleavage of a peptide by degradation of methionine.

Wikipedia,2013

AHL signals in Gram-ve bacteria
Structure of acyl-HSLs

- The family of N-Acyl homoserine lactones (AHL/ASL) seem to be an almost universal signal factor in Gram negatives.
- Because in the most well-studied systems the autoinducers (AIs) were N-acylated derivatives of L-homoserine lactone (acyl-HSL).
- Signal specificity of the acyl side-chain is conferred by:

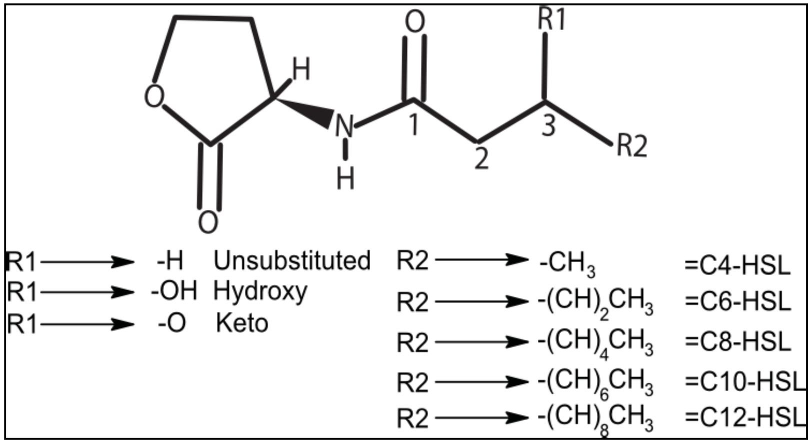
1. An acyl chain C-3 substituent (oxo or hydroxy), or
2. The length of the N-acyl side chain.

AHL signals in Gram-ve bacteria

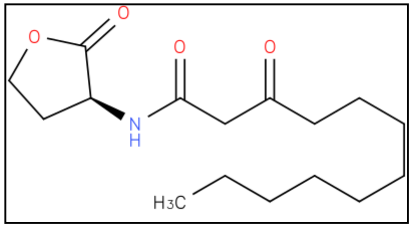
Structure of Acyl homoserine lactones (AHLs)

1. R1 group is defined as substitutions on the third carbon, and
2. R2 group is defined as acyl chain length.

- The acyl chains can vary with even carbon numbers starting from C4, C6, C8 to up to 12 carbons or even more.
- Odd number of carbons in the side chains has also been reported.



Usual substitutions are on the third position (R1 in Figure 3) with hydrogen (un-substituted), a keto (3-oxo), or a hydroxy (3-OH).

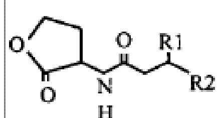


N-(3-oxododecanoyl)-L-homoserine lactone.

Annous *et al*.,2009

AHL signals in Gram-ve bacteria
Structure and functions of AHLs

- Acyl side-chain length and the substitutions on the side chain provide signal specificity.
- Acyl side chains of the signals can be fully saturated, they can have hydroxyls (OH) or carbonyls (C=O) on the third carbon, and they can have lengths of 4 to 16 carbons.
- R_1= –H, –OH or =O;
- R_2= $-CH_3$, $-(CH_2)_{2-14}CH_3$ or $-(CH_2)_5CH{=}CH(CH_2)_5CH_3$.

Structure	R: R1	R: R2	AHL molecule	Bacteria	Behaviour
 The general structure of AHLs (acyl-HSLs)	H	H	C4-HSL	*S. liquefaciens*	Cell motility/swarming
	OH	H	3-hydroxy-C4-HSL	*V. harveyi*	Bioluminescence
	H		C6-HSL	*C. violaceum*	Pigments/antibiotics/chitinase
	O		3-oxo-C6-HSL	*E. carotovora* *P. aureofaciens*	Pathogenicity/antibiotics Biocontrol activity/antibiotics
	H		C7-HSL	*R. leguminosarum*	?
	O		3-oxo-C8-HSL	*A. tumefaciens*	Conjugation
	OH		3-hydroxy-C14:1-HSL	*R. leguminosarum*	Growth inhibition/rhizosphere genes

Fray,2001;..

The names and abbreviations of some signaling molecules (compounds) in bacteria

- C4-HSL, N-butanoyl-L-homoserine lactone;
- C6-HSL, N-hexanoyl-L-homoserine lactone;
- C7-HSL, N-heptanoyl-L-homoserine lactone;
- C8-HSL, N-octanoyl-L-homoserine lactone;
- C10-HSL, N-decanoyl-L-homoserine lactone;
- C12-HSL, N-dodecanoyl-L-homoserine lactone;
- C14-HSL, N-tetradecanoyl-L-homoserine lactone;
- 3-oxo-C6-HSL, N-(3-oxohexanoyl)-L-homoserine lactone;
- 3-oxo-C8-HSL, N-(3-oxooctanoyl)-L-homoserine lactone;
- 3-oxo-C10-HSL, N-(3-oxodecanoyl)-L-homoserine lactone;
- 3-oxo-C12-HSL, N-(3-oxododecanoyl)-L-homoserine lactone;
- 3-oxo-C14-HSL, N-(3-oxotetradecanoyl)-L-homoserine lactone.

Numerical multiplier (or multiplying affix

Number	Multiplier	Number	Multiplier
1	mono-	32	dotriaconta-
2	di-	40	tetraconta-
3	tri-	50	pentaconta-
4	tetra-	60	hexaconta-
5	penta-	70	heptaconta-
6	hexa-	80	octaconta-
7	hepta-	90	nonaconta-
8	octa-	100	hecta-
9	nona-	200	dicta-
10	deca-	300	tricta-
11	undeca-	400	tetracta-
12	dodeca-	500	pentacta-
13	trideca-	600	hexacta-
14	tetradeca-	700	heptacta-
15	pentadeca-	800	octacta-
16	hexadeca-	900	nonacta-
17	heptadeca-	1000	kilia-
18	octadeca-	2000	dilia-
19	nonadeca-	3000	trilia-
20	icosa-	4000	tetralia-
21	henicosa-	5000	pentalia-
22	docosa-	6000	hexalia-
23	tricosa-	7000	heptalia-
30	triconta-	8000	octalia-
31	hentriconta-	9000	nonalia-

AHL signals in Gram-ve bacteria
Diffusibility of the acyl-HSLs

- The diffusion speed is correlated with the nature of the acyl chain and long acyl chain AHLs, if they can diffuse at all, would diffuse more slowly than short acyl chain AHLs.

1. Short-chain signals such as C4-C6 HSLs (detected by biosensor *C. violaceum* CV026) diffuse freely through the cell membrane.
2. Long chain signals such as 3-oxoC12-HSL(detected by biosensor *A. tumefaciens* NTL4 (pZLR4) partitions into cells, presumably in the membrane.
3. Signals like 3-oxoC12-HSL can diffuse into the surrounding environment but export is enhanced by the mexAB-oprM (a multi-component channel) and perhaps other, efflux pumps.

Parsek and Greenberg,2000;Nagy,2010

Multilingual bacteria
Multiple QS signals

- The presence of multiple QS signals in culture supernatants of gram-negative bacteria is not a rare finding. E.g.

1. *Pseudomonas aeruginosa*
2. *Pseudomonas aureofaciens*
3. *Pseudomonas putida*

González *et al.*,2009

Multilingual bacteria

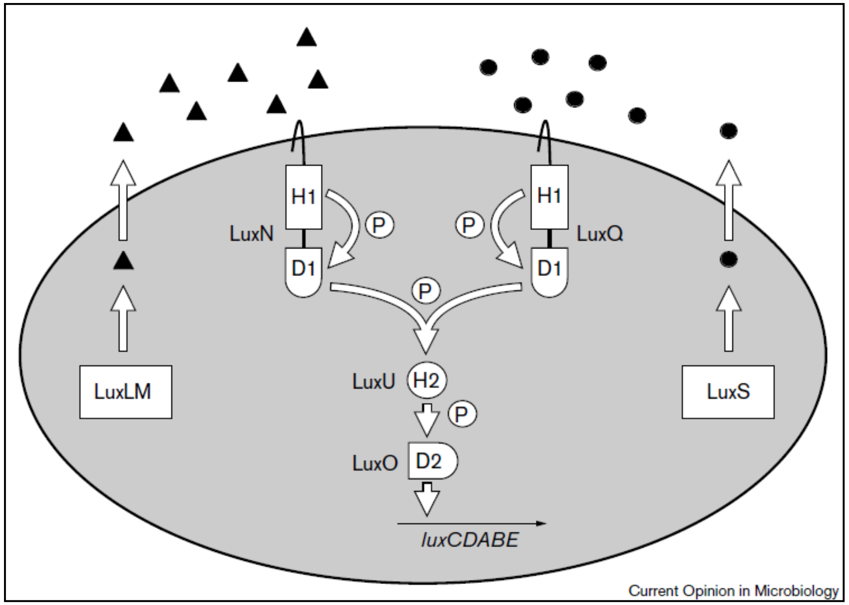
Two QS systems

Vibrio harveyi

- The two quorum sensing circuits of *V. harveyi* are shown.

1. **Autoinducer 1 (AI-1):** A HSL autoinducer (triangles).
2. **Autoinducer 2 (AI-2):**

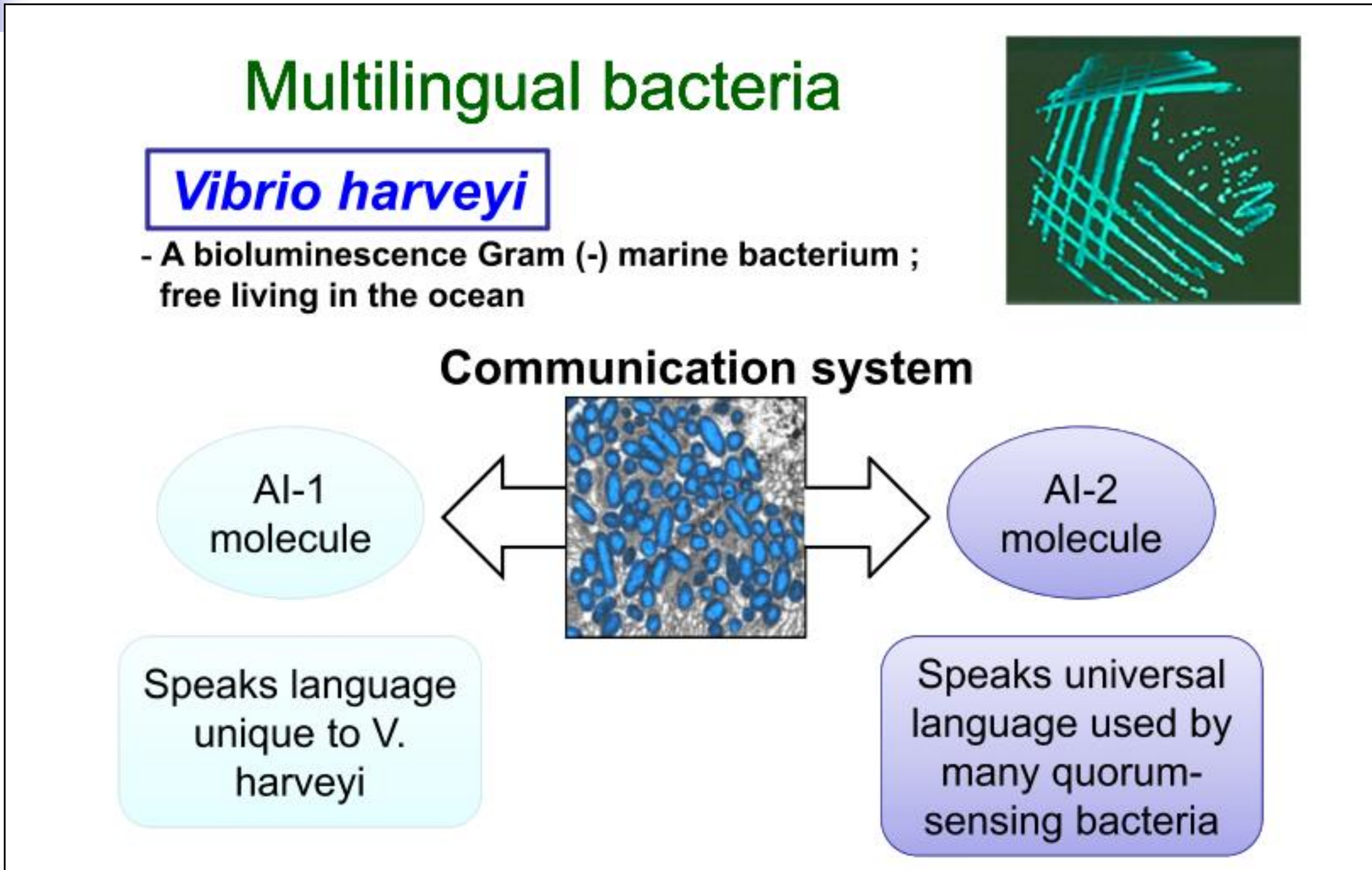
- Synthesis of AI-1 and AI-2 is dependent on LuxLM and LuxS, respectively.



Bassler,1999

Different AHLs synthases have been described in different quorum sensing signalling pathways. For instance, in *V. harveyi* a 3-hydroxy-C4-HSL is synthesized by the LuxM synthase and received by luxN protein. The genes coding for these proteins show no homology to the previously described LuxR/I quorum sensing system.

Multilingual bacteria
Two QS systems
Vibrio harveyi



Haung,2011

Multilingual bacteria
Multiple QS signals

- *Pseudomonas aeruginosa*, in which quorum sensing systems have been extensively characterized, two main AHSLs are produced:

1. C4-HSL, and
2. 3oxo-C12-HSL.

- While the same signaling systems can synthesize other AHSLs in smaller amounts:

3. 3-oxo-C6-HSL, and
4. C6-HSL.

González *et al.*,2009

Quorum sensing
LasI-R and RhlI-R systems
Pseudomonas aeruginosa

- Both C4-HSL and 3-oxo-C_{12}-HSL signaling molecules diffuse out into the environment and, upon reaching a threshold concentration, activate receptors *lasR* and *rhlR*.

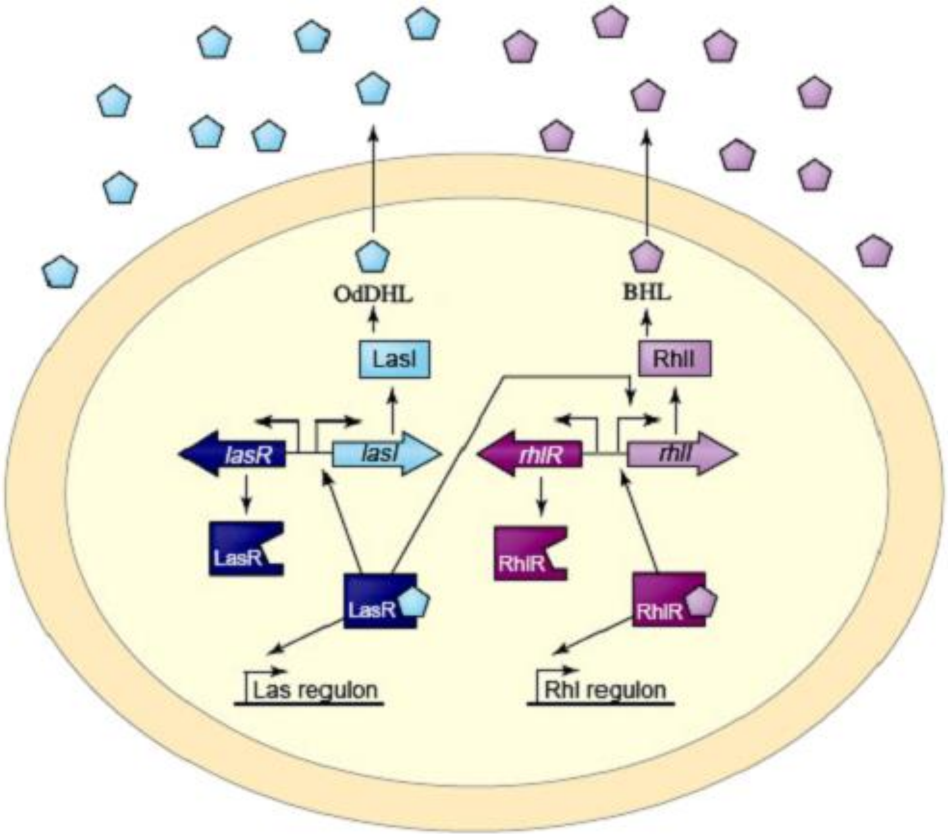
1. The virulence factors LasA (staphylolytic protease) and LasB (elastase) are thought to be under control of the lasI/R system, whereas
2. Pyoverdin is believed to be under rhlI/R control.

Adonizio,2008

Quorum sensing

LasI-R and RhlI-R systems

Pseudomonas aeruginosa



Either LasR or RhlR can bind with varying efficiency to any *lux*-box-like element.

Adonizio,2008

Multilingual bacteria
Multiple QS signals

- *In Pseudomonas putida*
- Main QS signals are:

1. 3-oxo-C10-HSL,and
2. 3-oxo- C12-HSL.

- Minor QS signals which are secreted in minor amounts are:

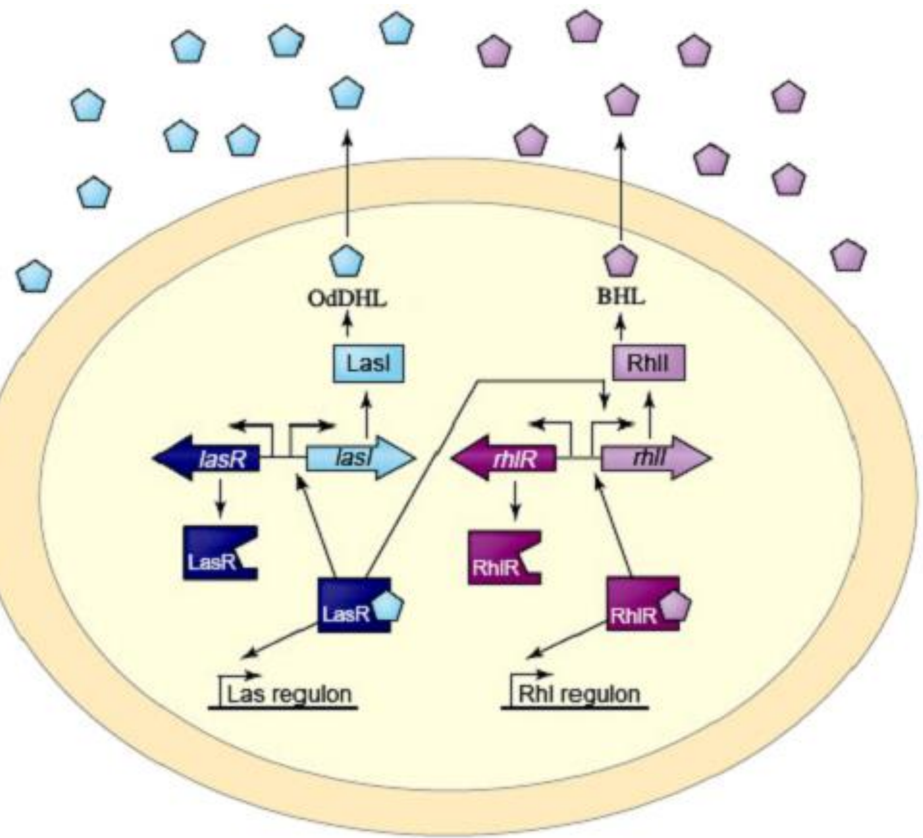
3. 3-oxo-C8-HSL, and
4. 3-oxo-C6-HSL.

González *et al*.,2009

Quorum sensing

LasI-R and RhlI-R systems

Pseudomonas aeruginosa



Either LasR or RhlR can bind with varying efficiency to any *lux*-box-like element.

Adonizio,2008

Isolation of *N*-acyl-homoserine lactone-producing bacteria

Media

Luria Broth and Agar Plates

- For 1,000-ml D.H_2O:
- 10 g of Tryptone
- 5 g of Yeast Extract
- 5 g of NaCl
- For agar plates add 15 g of agar

Pierson III, 2005

Alternative composition for Luria-Bertani (LB) agar:
1% w/v peptone, 0.5% w/v yeast extract, 0.5% w/v NaCl, 1.5% agar in 100 mL distilled water.

Isolation of *N*-acyl-homoserine lactone-producing bacteria
Media

Phosphate Buffered Saline (PBS)

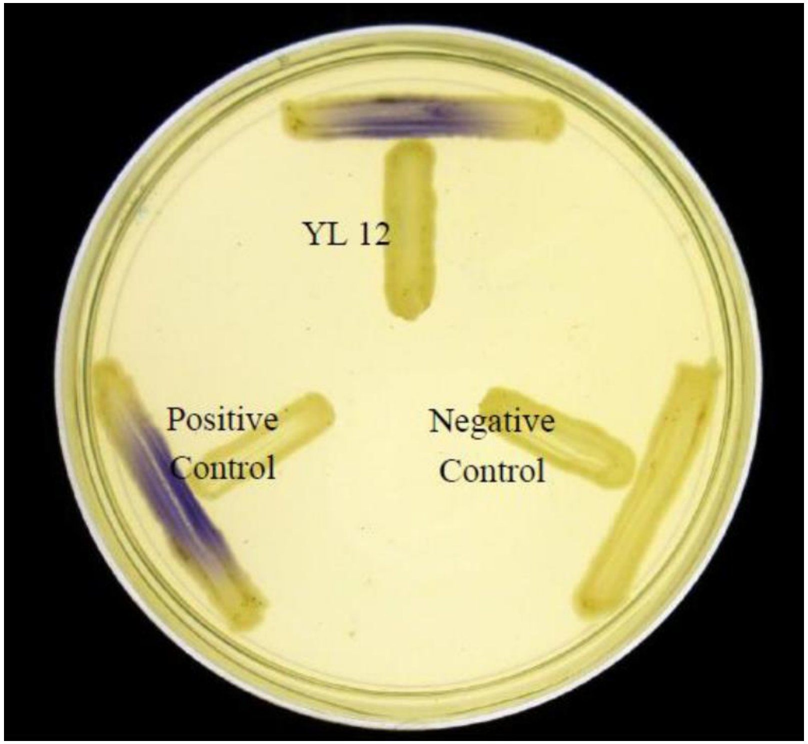
- Per 1000-ml:
- 1.6 g of NaCl
- 0.2 g of KH_2PO_4
- 2.9 g of Na_2HPO_4-$12H_2O$
- 800 ml of dionized H_2O
- Adjust pH to 7.4
- Adjust final volume to 1000 ml.

Pierson III, 2005

Quorum Sensing

Cross-streaking of *Aeromonas caviae* strain YL12 with *C. violaceum* CV026

- Cross-streaking of strain YL12 with *C. violaceum* CV026 was observed to trigger violacein production in the biosensor.
- *E. carotovora* GS101 and *E. carotovora* PNP22 were used as positive and negative controls, respectively.
- In the cross-streaking experiment conducted, strain YL12 triggered CV026 violacein production, which suggests that YL12 produces diffusible short chain AHLs.



Lim *et al.*,2014

AHL screen assay
Pectobacterium sp.

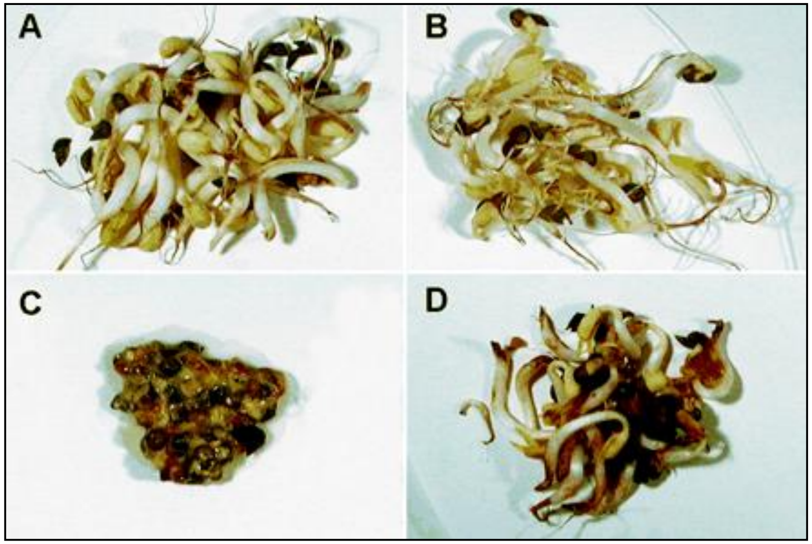
- Appearance of bean sprouts, where the soaking water has been inoculated with different bacterial strains to determine their spoilage potentials.

A. Control (uninoculated);

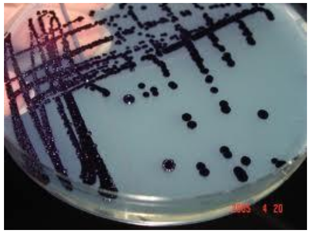
B. Bean sprouts inoculated with the nonspoiling strain C1JM;

C. Bean sprouts inoculated with the spoiling strain *Pectobacterium* sp. strain A2JM;

D. Bean sprouts inoculated with the AHL-deficient A2JM *luxI* mutant.



Chromobacterium violaceum
Colony morphology



- It is a Gram-negative, facultative anaerobic, non-sporing coccobacillus.
- It is part of the normal flora of water and soil of tropical and sub-tropical regions of the world.
- It produces a natural antibiotic called violacein.
- It grows readily on nutrient agar, producing distinctive smooth, low convex, circular, with an entire margin colonies with a dark violet metallic sheen (due to violacein production).

Thin layer chromatography (TLC)

Thin layer chromatogram showing the range of AHLs produced by various bacteria

- TLC is a powerful technique both for analysis and separation of different AHLs, that can be analysed either by biological methods or analytical techniques.
- Extracts are spotted on TLC plates and allowed to run in a mixture of organic solvents.
- Plates are usually C18 silica matrix plates.
- Separated AHLs are either visualized with UV or by chromic agents like potassium dichromate in sulphuric acids.

Thin layer chromatography (TLC)

Thin layer chromatogram showing the range of AHLs produced by various bacteria

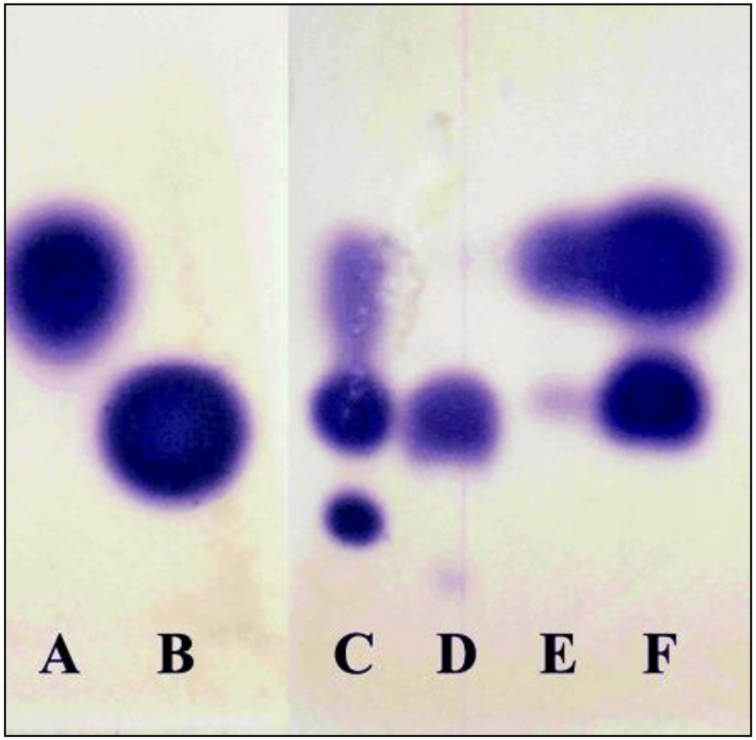
- Alternatively, spots could be scraped off and the material obtained extracted with either dichloromethane or ethyl acetate for further analysis.
- One disadvantage is that:

1. No structural information can be obtained, and
2. Interference of metabolites is hard to avoid.

- For example two unknown spots found in a report which could not be verified.

Thin layer chromatogram showing the range of AHLs produced by various *Erwinia carotovora* species

- The sensitivity of the *Chromobacterium violaceum* bioreporter (indicator) strain/a biosensor sensitive to short-chain AHLs) varies according to the AHL being detected.
- Thus the intensity of pigment is not a direct indicator of the relative abundance of each AHL.

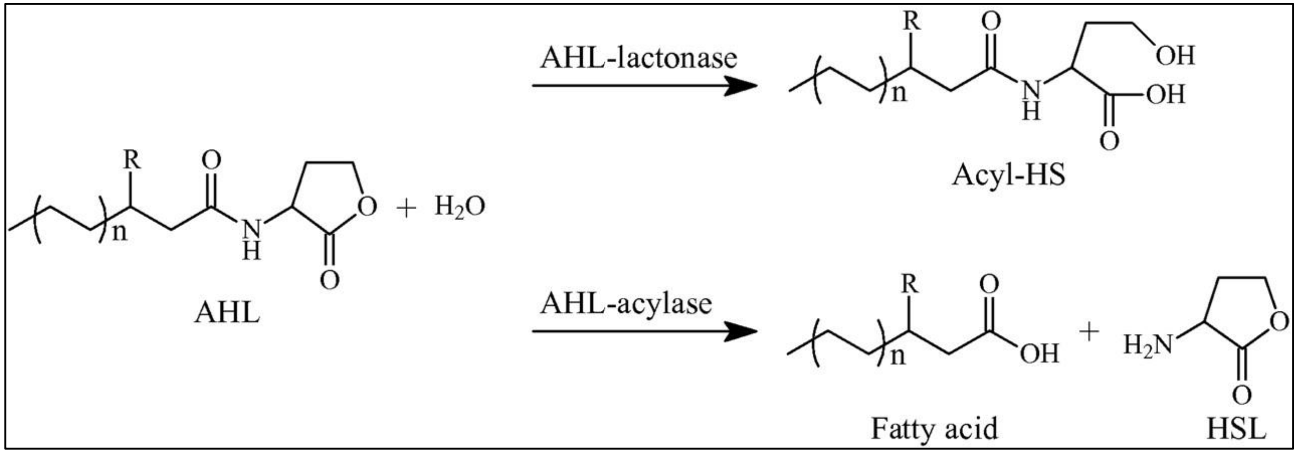


Fray,2001;..

Mechanisms of QS inhibition

Two main types of AHL-degrading enzymes

1. AHL lactonases hydrolyze the lactone ring in the homoserine moiety of AHLs, without affecting the rest of the signal molecule structure.
2. AHL-acylases break the amide linkages of the AHLs (amidohydrolase).



Wang *et al.*,2004

Mechanisms of QS inhibition

Bacterial enzymes as quorum sensing inhibitors

Source of quorum sensing inhibitor	Enzyme	Degraded quorum sensing signal
Bacillus sp.strain 240B1	Lactonase	AHLs
Bacillus thuringiensis	Lactonase	AHLs
Stappia[a] sp. strains 5, 176 and 97-1	Lactonase	AHLs
Oceanobacillus strains 30, 172, and 97-2	Lactonase	AHLs
Halomonas sp. strain 33	Lactonase	AHLs
Tenacibaculum discolor strain 20J	Acylase/Lactonase	AHLs
Hyphamonas sp. DG895	Acylase/Lactonase	C4HSL and 3OC12-HSL
Alteromonas sp. strain 168	Acylase	C4HSL and 3OC12-HSL
Bacillus megaterium	AHL-oxidase	C4HSL and 3OC12HSL
Bacillus circulans strain 24	Different from Lactonase[b]	C4HSL and 3OC12HSL
Bacillus pumilus S8-07	AHL-acylase	3OC12HSL
Ralstonia sp. XJ12B	AHL-acylase	Long chain AHLs
Pseudomonas aeruginosa PAO1	AHL-acylase	Long chain AHLs
Rhodococcus erythropolis strain W2	AHL-Lactonase	AHLs
	Acylase (Amidohydrolase)	AHLs
	Oxidoreductase activity	3-oxo-N-AHLs
Burkholderia strain GG4	AHL – oxidoreductase	3OC6HSL
Agrobacterium tumefaciens	AHL-Lactonase	AHLs
Arthrobacter sp. IBN110	AHL-Lactonase	AHLs
Acinetobacter sp. strain C1010	Lactonase	AHLs

Kalia,2013

Mechanisms of QS inhibition

QQ bacteria with AHL-acylase activity

AHL acylase		
AiiD	*Ralstonia eutropha*	C8-12-HSL
PvdQ	*Pseudomonas aeruginosa*	C7-12-HSL with or without C3-substitution
QuiP	*Pseudomonas aeruginosa*	C7-14-HSL with or without C3-substitution
AiiC	*Anabaena* sp. PCC 7120	Chain length more than C10
AhlM	*Streptomyces* sp. M664	Chain length more than C8
Aac	*Ralstonia solanacearum*	Chain length more than C6
	Shewanella sp. MIB015	Broad but prefer long chain
HacA	*Pseudomonas syringae*	C8,C10, C12-HSL
HacB	*Pseudomonas syringae*	C6-12-HSL with or without C3-substitution
	Variovorax sp.	Broad
	Variovorax paradoxus	Broad
	Tenacibaculum maritimum	C10-HSL
	Comomonas sp. D1	C4-16-AHL with or without C3-substitution
	Rhodococcus erythropolis W2	C10-HSL

Chen *et al.*,2013

Manipulation of QS

Phytochemicals with proved antiquorum sensing activity

Eukaryotic disruption of bacterial QS/biofilm

Molecules
Gamma aminobutyric acid (GABA)
Pyrogallol
Curcumin
Cynnamaldheyde
Furocoumarins
Flavanones, flavonoids, flavonols
Ursolic acid
Rosmarinic acid
Salycilic acid

Molecules
Epigallocatechin gallate, Ellagic acid, Tannic acid
Urolithin A and B
4,5-*O*-dicaffeoyl quinic acid
Chlorogenic acid, vanillic acid, proanthocyanidins
Volatile organic compounds
Furanones

Nazzaro *et al.*,2013

Methods

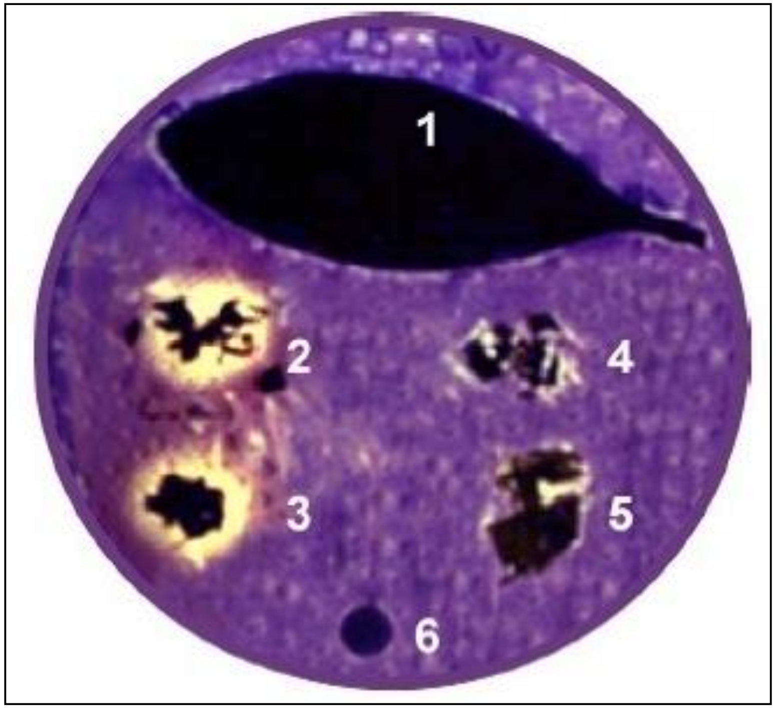
Preliminary screening of *Conocarpus erectus* for anti-QS activity using *Chromobacterium violaceum*

- Preliminary screening of *Conocarpus erectus* for anti-QS activity using *Chromobacterium violaceum* biomonitor strain.
- A yellowish "halo" of bacteria indicates an anti-QS effect.
- The samples were:

1. Washed whole leaf,
2. Washed macerated leaves, and
3. Unwashed macerated leaves of fresh *Conocarpus erectus*.

- Negative controls:

4. Washed,
5. Unwashed leaves of *Hamelia patens*, and
6. Sterile paper disk were included.

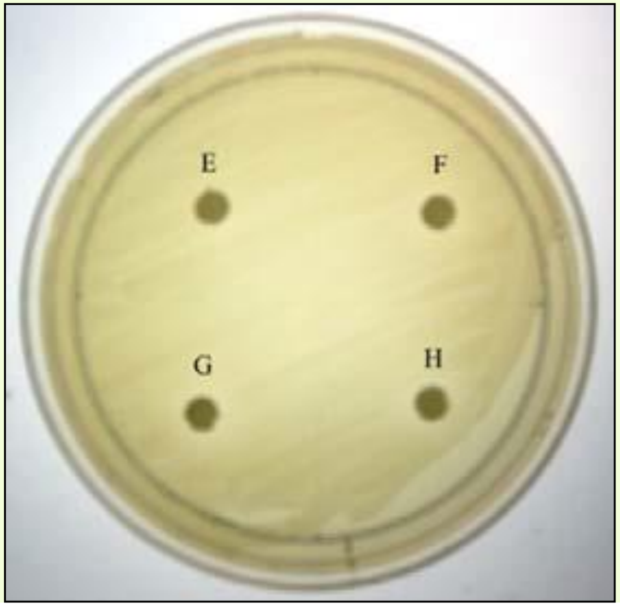


Adonizio *et al.*,2006 and 2008

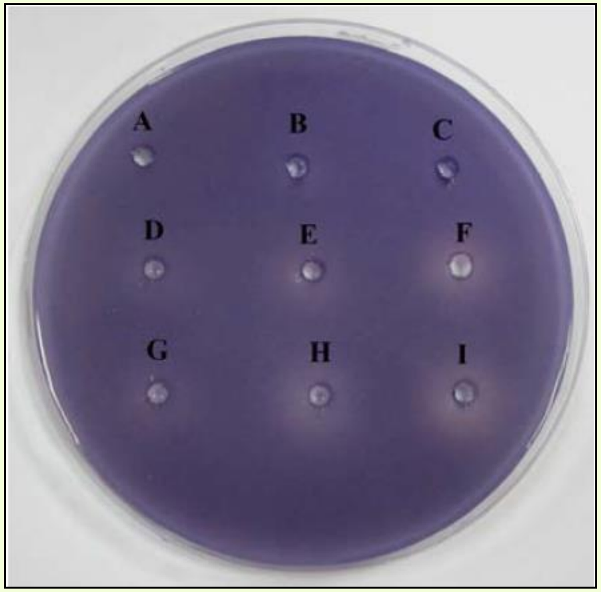
Antimicrobial vs. Antiquorum sensing assay

It is important to ensure any anti-QS effect is not resulted from antibacterial activity. A-D controls

- **Antimicrobial assay**
- No antibacterial activity was detected (E-F) against CV with caffeine at different conc.



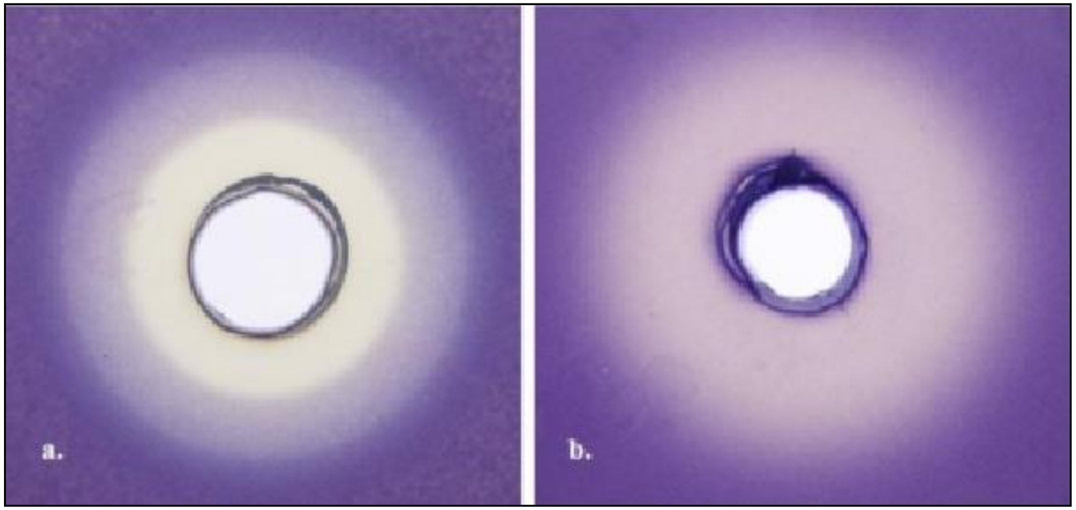
- **Antiquorum sensing assay**
- Caffeine at different conc. promoted QS inhibitory effect on CV.



Norizan *et al.*,2013

Anti-QS activity

Bioassay for both anti-qouorum- sensing and antibacterial sensing activities



Bioassay of TCM extracts using *Chromobacterium violaceum* CV026 showed both anti-quorum sensing and antimicrobial activities.

a. *Lilium brownii* (bai he) extract showing different active principles, i.e.

- antibacterial activity (inner clear ring), and
- QSI or quorum-sensing inhibition (outer creamy ring).

b. *Panax pseudoginseng* (ren shen) extract exhibited only QSI (creamy ring) and no antibacterial activity.

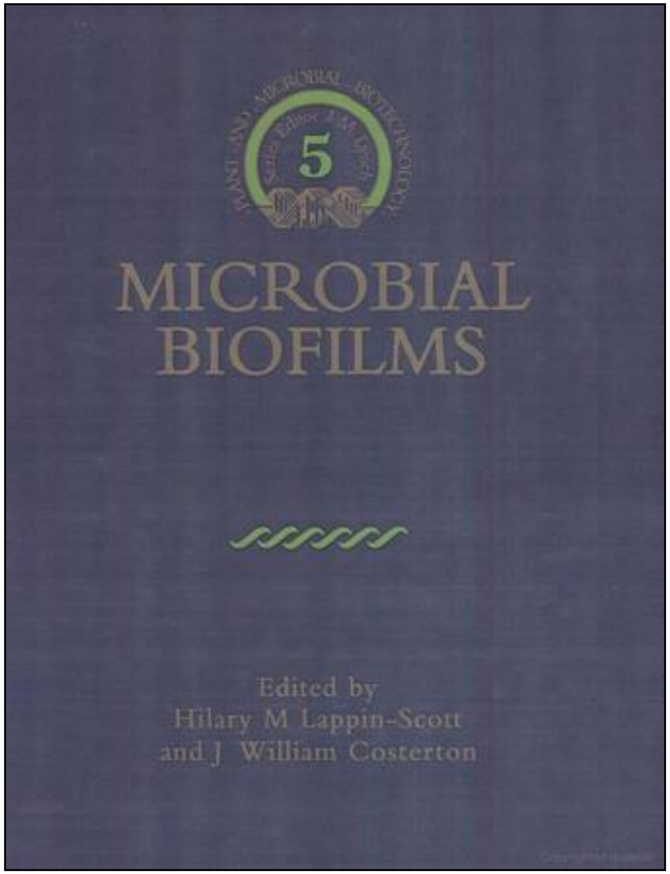
Yeo and Tham,2011

Microbial biofilms
City of Microbes

Key to understanding and controlling bacterial growth

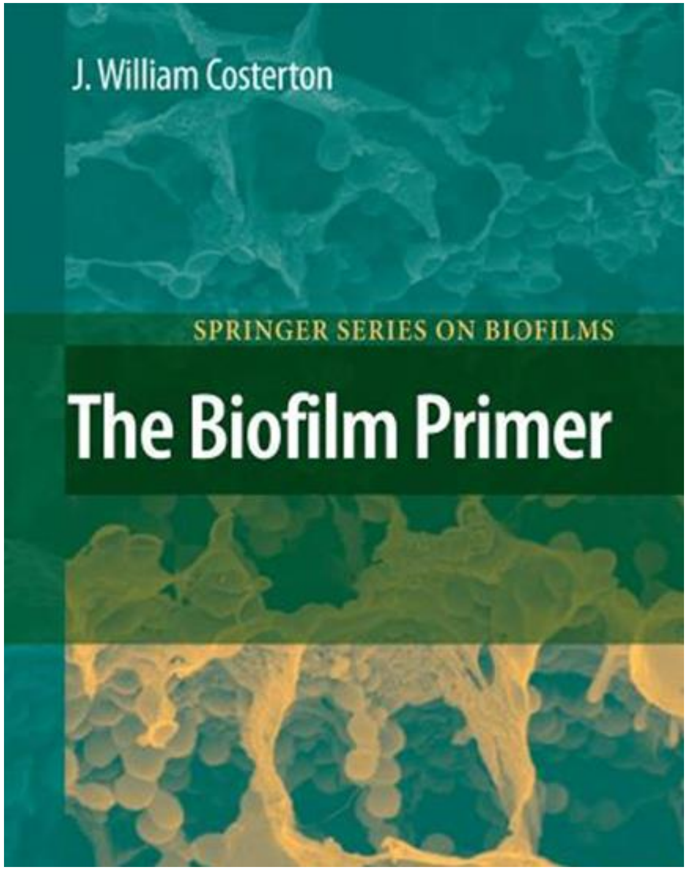
Microbial Biofilms

- **Microbial Biofilms**
- Editors: Hilary M. Lappin-Scott and J. William Costerton
- Cambridge University Press
- 2003
- 328 pages.



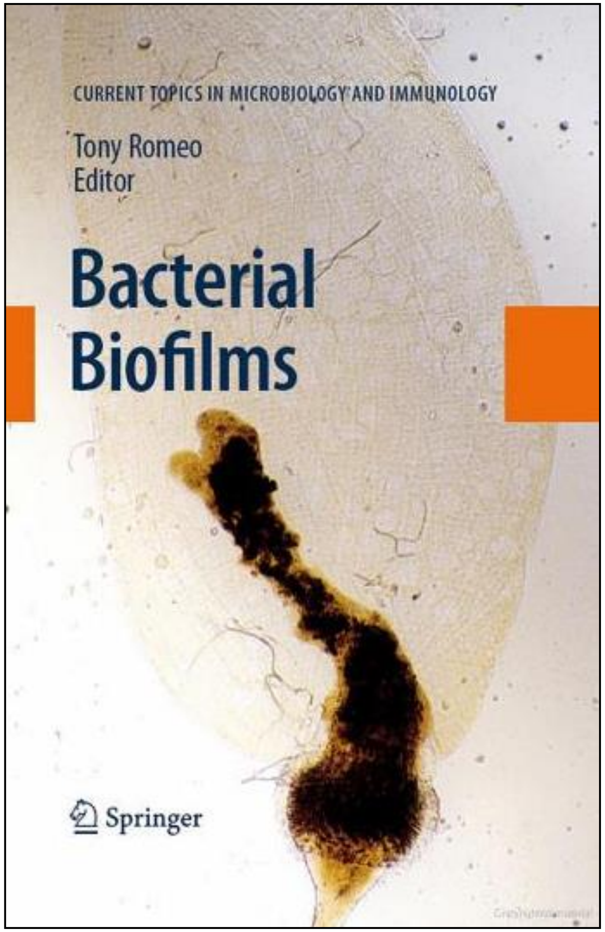
The Biofilm Primer

- **The Biofilm Primer**
- Author: J. William Costerton
- Springer
- 1 edition
- April 19, 2007
- 207 pages.



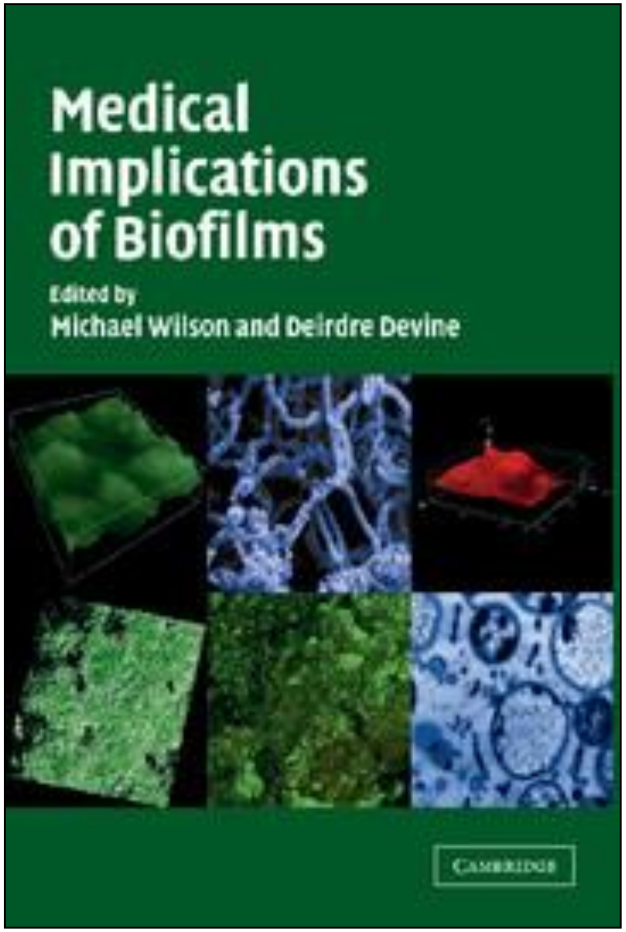
Bacterial Biofilms

- **Bacterial Biofilms**
- Current Topics in Microbiology and Immunology, Vol. 322.
- Editor: Tony Romeo
- Springer, 2008.
- 308 pages.
- Pdf 3.2 Mb.



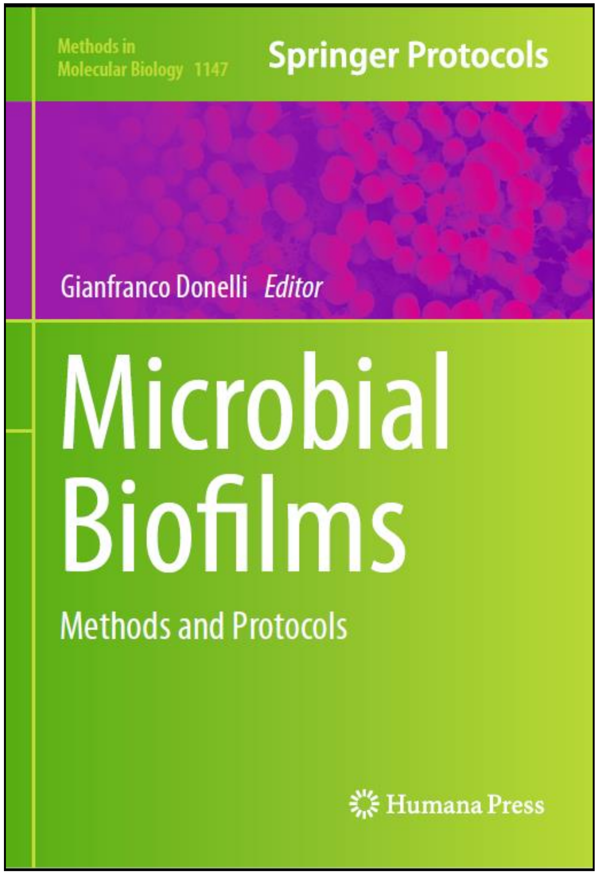
Medical Implications of Biofilms

- **Medical Implications of Biofilms**
- Michael Wilson and Deirdre Devine
- Publisher: Cambridge University Press.
- Cambridge books online
- Date Published: 2011
- 314 pages.



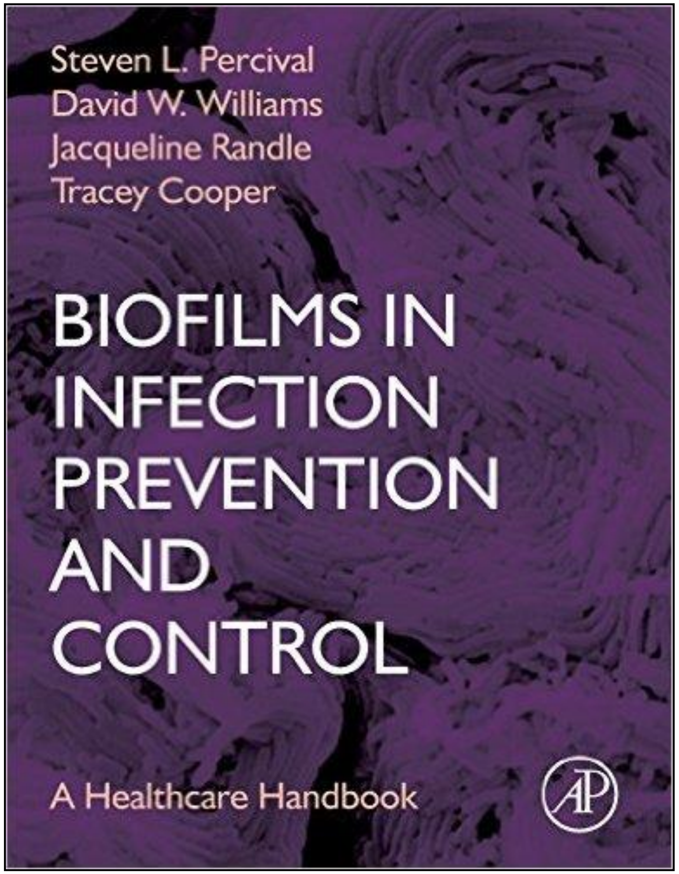
Microbial Biofilms
Methods and Protocols

- **Microbial Biofilms- Methods and Protocols**
- Editor: Gianfranco Donelli
- Publisher: Humana Press.
- Date Published: 2014
- 380 pp.



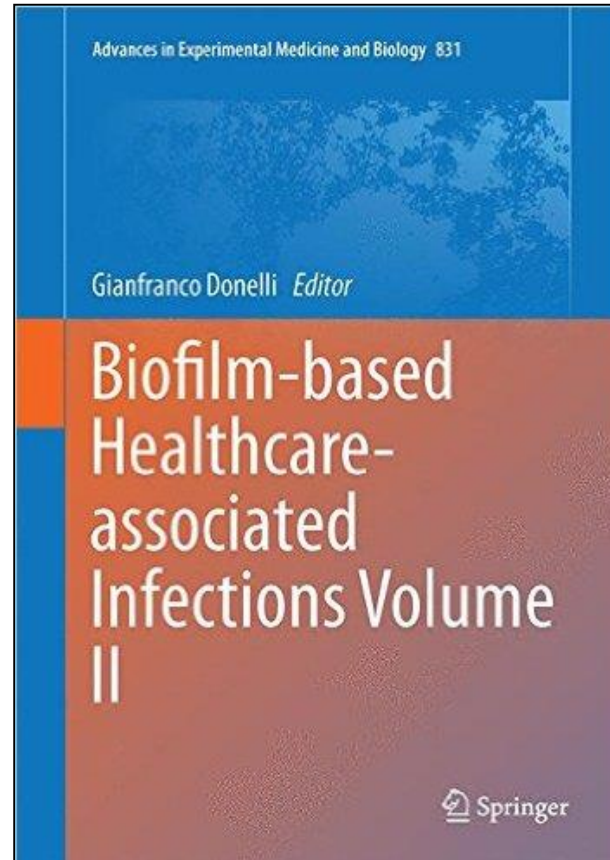
Biofilms in Infection Prevention and Control: A Healthcare Handbook 1st Edition

- Biofilms in Infection Prevention and Control: A Healthcare Handbook
- Steven L. Percival (Editor), David Williams BSc (Hons) PhD (Editor), Tracey Cooper (Editor), Jacqueline Randle (Editor)
- Hardcover: 394 pages
- Publisher: Academic Press; 1 edition (March 12, 2014)



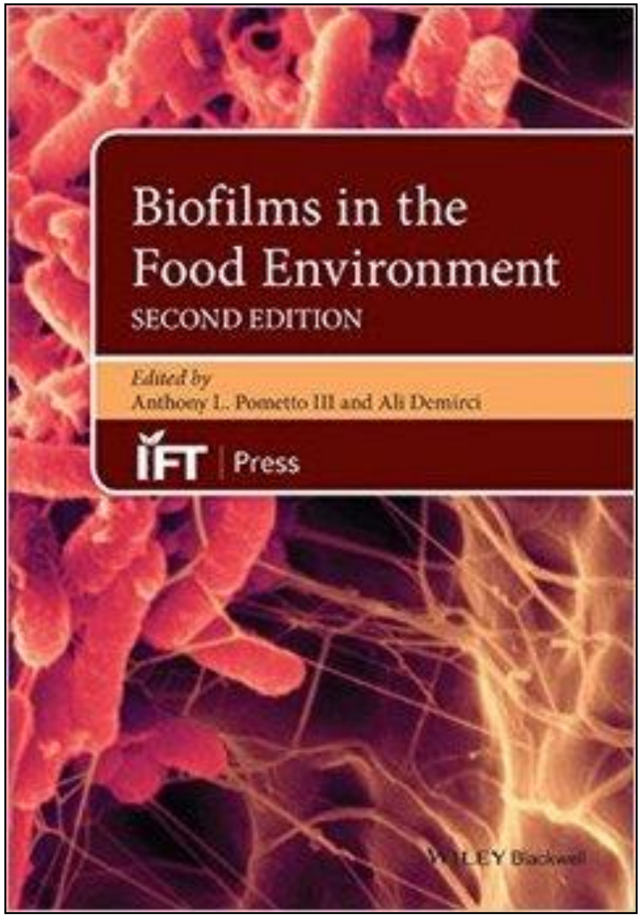
Biofilm-based Healthcare-associated Infections: Volume II (Advances in Experimental Medicine and Biology)

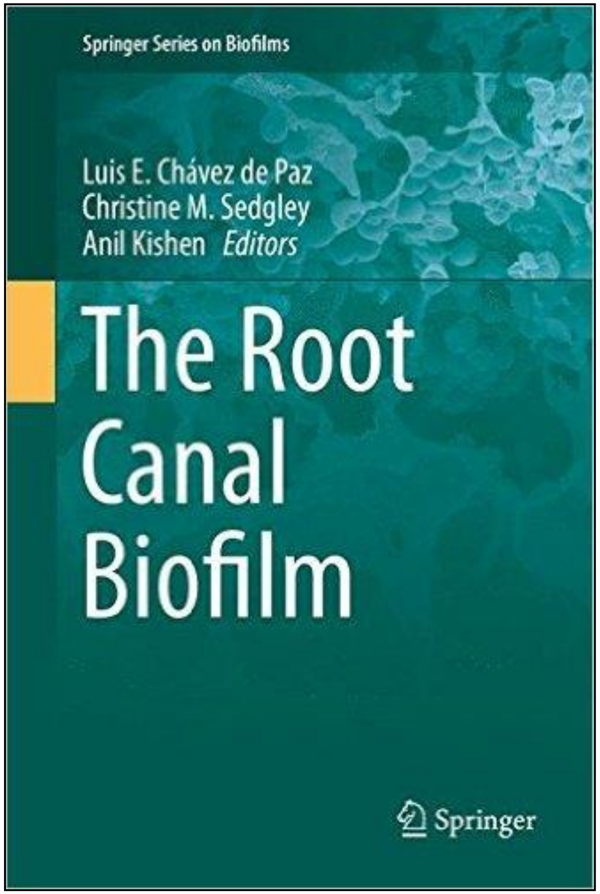
- Biofilm-based Healthcare-associated Infections: Volume II (Advances in Experimental Medicine and Biology)
- Editor Gianfranco Donelli
- Publisher: Springer; 2015th Edition.
- Hardcover: 195 pages



Biofilm

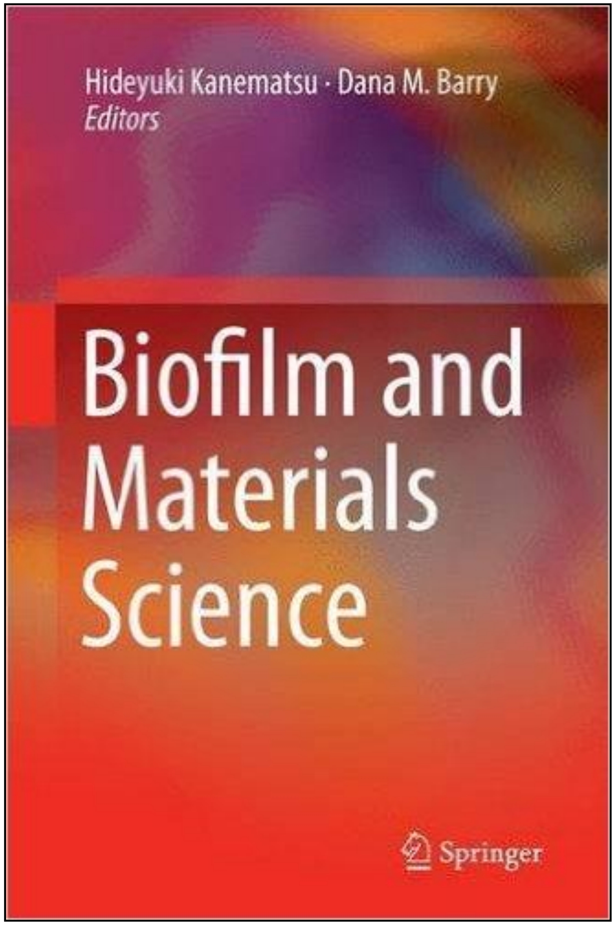
Books,2015

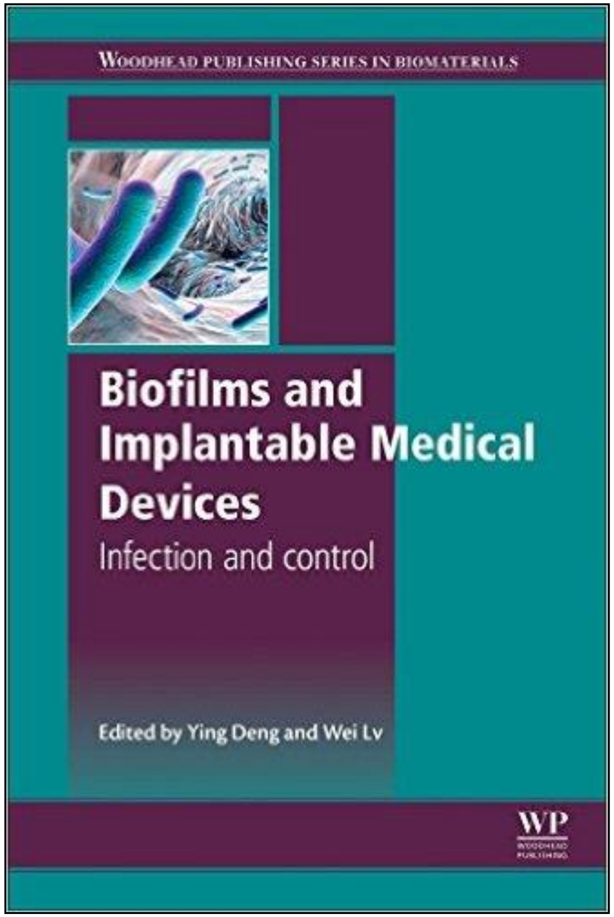




Biofilm

Books,2016





Bacterial autoaggregation and biofilm development, and their relationship with plant colonization

- Microbes' propensity (tendency) is:

1. to attach to both living and inanimate surfaces;
2. improves their likelihood of survival and proliferation.

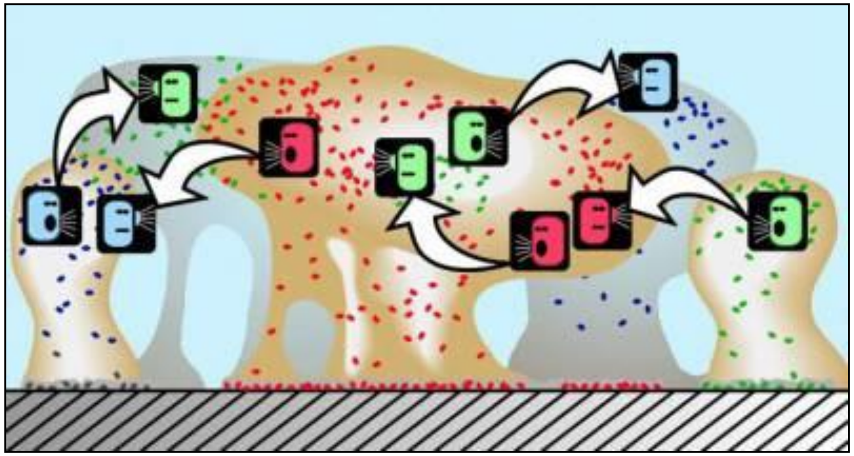
- They produce a sticky material that binds them together and anchors them to the surface forming dense, complex colonies of microorganisms known as biofilm.

Reiff,2013

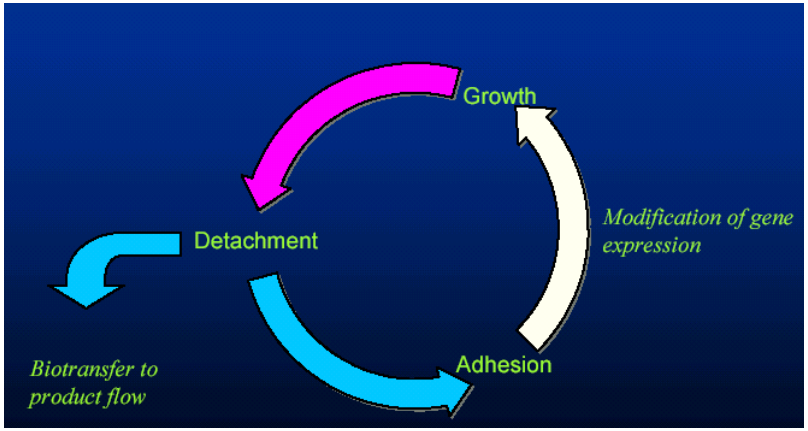
Biofilm

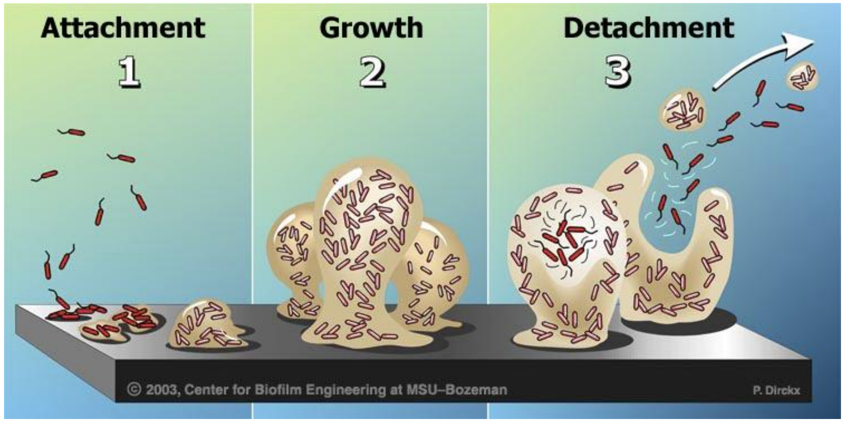
Quorum sensing

- Quorum sensing Sessile cells in a biofilm “talk” to each other via quorum sensing to build microcolonies and to keep water channels open.
- Source: Center for Biofilm Engineering, Montana State University-Bozeman



Biofilm life cycle



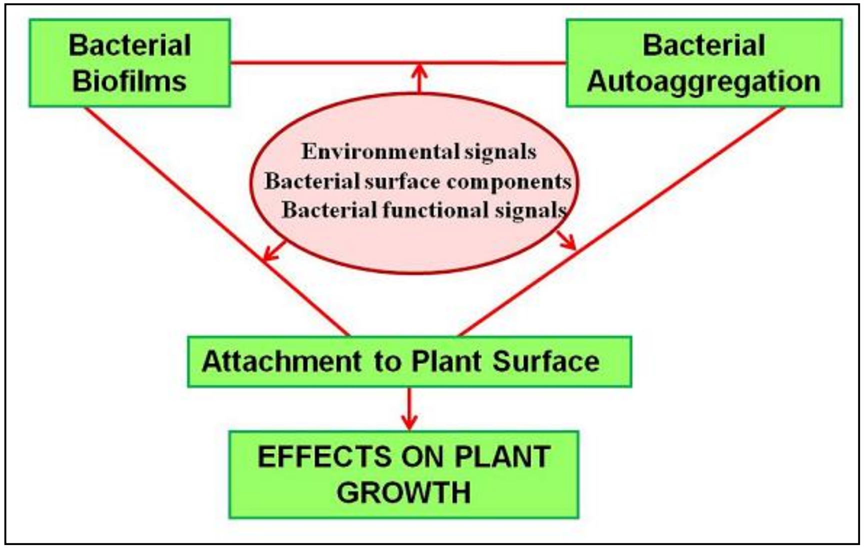


mpkb.org,2014;..

Bacterial autoaggregation and biofilm development, and their relationship with plant colonization

- Cell aggregation and biofilm formation in plant-bacterial associations are regulated by:

1. Environmental signals,
2. Nutrient limitation of growth,
3. Quorum sensing,
4. EPSs,
5. Flagella,
6. LPSs, and
7. Other factors.



Bogino *et al.*,2013

Common biofilm forming human bacterial

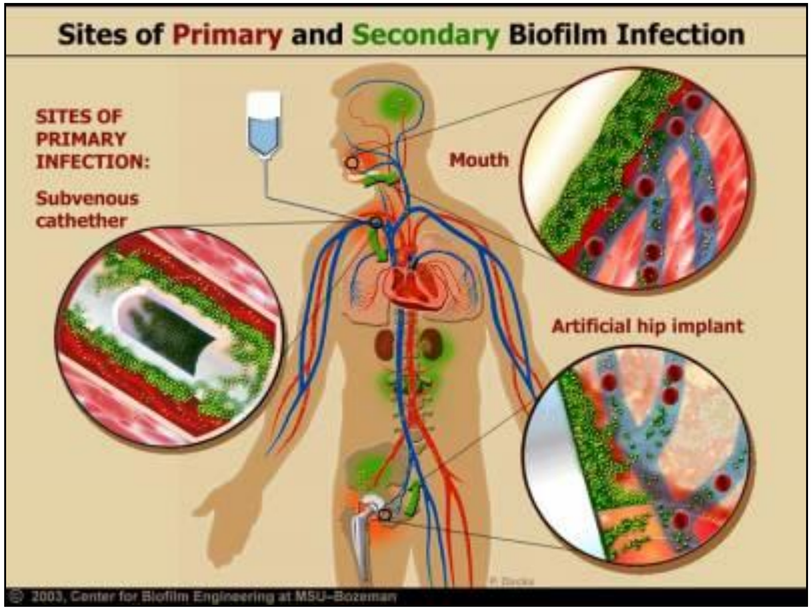
- A greater understanding of bacterial biofilm is required for the development of novel, effective control strategies thus resulting improvement in patient management.
- *Escherichia coli*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Stereptococcus epidermidis*
- *Enterobacter cloacae*
- *Klebsiella pneumoniae*

Jamal *et al.*,2015

Biofilm

Common sites of biofilm infection

- Once biofilm reach the bloodstream they can spread to any moist surface of the human body.
- Source: Center for Biofilm Engineering, Montana State University-Bozeman.



Production, composition, and functional roles of exopolysaccharides (EPSs) in beneficial and pathogenic plant-associated bacteria

Bacteria/plant association	Exopolysaccharide	Chemical composition	Function
B. japonicum symbiosis with *Glycine max*	EPS [100,101]	Pentasaccharide units (mannose:galacturonic acid:glucose:galactose 1:1:2:1)	Biofilm formation on both inert and biotic surfaces. Roles during the early stages of interaction with the host plant (initial attachment of rhizobia to root epidermal cells) [102]
M. tianshanense symbiosis with *Glycyrrhiza uralensis*	EPS	ND	Involved in biofilm formation and successful establishment of symbiosis [103]
A. tumefaciens ubiquitous plant pathogen	Succinoglycan [104]	See above	Increased production of succinoglycan results in reduced attachment and biofilm formation [105]
X. fastidiosa plant pathogen	Putative Fastidian gum [106]	Putative tetrasaccharide units (glucose-1-phosphate, glucose, mannose, and glucuronic acid)	Possibly involved in bacterial pathogenicity [106] Cell attachment and overall biofilm formation [107]
X. campestris *X. axonopodis* plant pathogens	Xanthan gum [108]	Pentasaccharide units (glucose:mannose:glucuronic acid 2:2:1 derivatized with acetyl and pyruvyl moieties)	Essential for microcolony formation [74] Formation of structured biofilms on abiotic surfaces and in infected [illegible]

Bogino *et al.*,2013

Production, composition, and functional roles of exopolysaccharides (EPSs) in beneficial and pathogenic plant-associated bacteria

Bacteria/plant association	Exopolysaccharide	Chemical composition	Function
P. stewartii plant pathogen	Stewartan [111]	Heptasaccharide units (glucose:galactose:glucuronic acid 3:3:1)	Essential for appropriate adhesion and for maturation of biofilm structure. Also a virulence factor required for effective host colonization and efficient dissemination through xylem vessels [112]
E. amylovora plant pathogen	Amylovoran [113]	Pentasaccharide units (galactose:glucose 4:1, and pyruvate residues)	Pathogenicity factor required for biofilm formation [114]
	Levan [115]	Homopolymer of fructose	Virulence factor. Also contributes to biofilm formation [114]
R. solanacearum plant pathogen	Acidic EPS I [116]	Putative structure composed by *N*-acetylgalactosamine and amino sugars (bacillosamine, galactosaminuronic acid)	Major virulence factor [117]

LMW: low molecular weight; HMW: high molecular weight; ND: not determined.

Bogino *et al.*,2013

Quorum sensing and biofilm

1. Quorum sensing control gene expression in groups of bacteria, and
2. Biofilms organized groups of bacteria.

- Bacteria often tend to attach to surfaces and form communities enmeshed in a self-produced polymeric matrix.
- These communities are called a biofilm.

Wikipedia,2010;...

Quorum sensing and biofilm

- Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species.
- As the dense and protected environment of the film allows them to cooperate and interact in various ways.

Wikipedia,2010;...

Quorum sensing and biofilm
Resistance to detergents and antibiotics

- One benefit of biofilm is increased resistance to detergents and antibiotics.
- This resistance to antibiotics in both stationary phase cells and biofilms may be due to the presence of persister cells (those cells tolerant to antibiotics).
- Many bacteria quickly develop into biofilm communities.
- These biofilms are quite resistant to antibiotic treatment.

Wikipedia,2010;...

Quorum sensing and biofilm

- In general, bacterial pathogens use quorum sensing to ensure that virulence genes are only expressed after their population has reached a critical size.
- This unified attack strategy makes it more difficult for the host to mount an effective defense.
- Quorum sensing has been shown to directly control:

1. Biofilm formation (A highly structured polymer matrices produced by sessile bacteria (bacteria living within a biofilm).
2. The expression of virulence genes,
3. Swarming,
4. Conjugation, and
5. Production of secondary metabolites.

Biofilms

Common examples

- Biofilms are a collection of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface.
- You are already familiar with some biofilms:

1. The plaque on your teeth,
2. The slippery slime on river stones,
3. The gel-like film on the inside of a vase which held flowers for a week.

- Thus, among infectious bacteria, talk is not cheap.
- Biofilm exists wherever surfaces contact water.

Biofilms

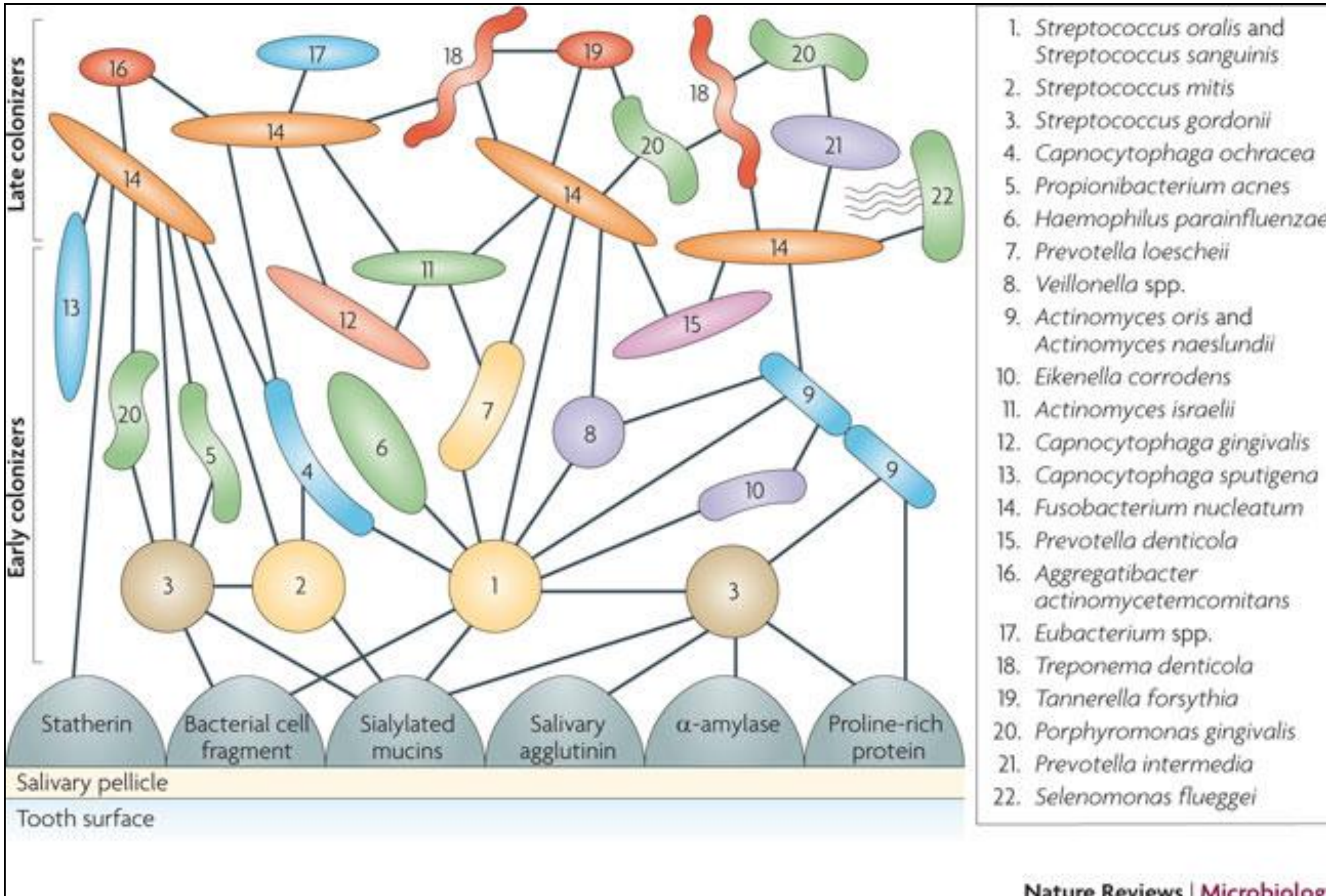
Biofilm in a water pipe

- The interiors of almost all water distribution systems eventually develop biofilms that may harbor pathogenic microbes and promote metal pipe corrosion, scaling and sediment buildup.
- Biofilms can discolor water or cause it to take on disagreeable tastes or odors.

Reiff,2013

Biofilms

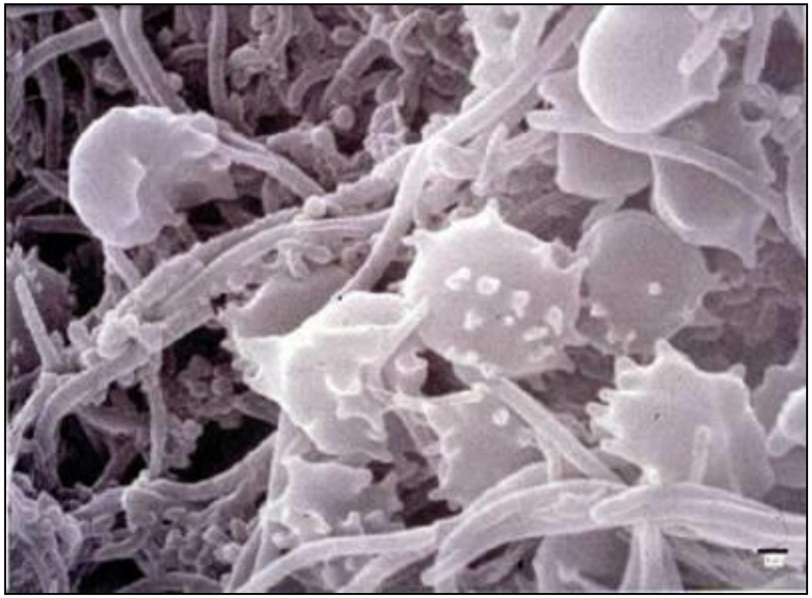
Bacterial species involved with dental biofilm accumulation and tooth decay. All employ quorum sensing



Giuliano,2013

Biofilm
Dental plaque

- Dental plaque is composed of more than 500 species.
- Over 500+ biofilm species colonize the human mouth, causing tooth decay and gum disease.
- This image, retrieved infected dental implant, was taken by a scanning electron microscope.



mpkb.org,2014

Biofilm

Multispecies/monospecies microbial community

- Biofilms can contain many different types of microorganism.
- e.g. bacteria, archaea, protozoa, fungi and algae; each group performing specialized metabolic functions.
- However, some organisms will form monospecies films under certain conditions.
- In most natural settings, bacteria grow predominantly in biofilms.
- Multiple bacterial species as opposed to the mono-species biofilms.

Wikipedia,2010;Schaechter,2004

Biofilm

Multispecies/monospecies microbial community

- Most biofilm studies examine mono-species cultures, whereas nearly all biofilm communities in nature comprise a variety of microorganisms.
- However, most natural biofilms are actually formed by multiple bacterial species.
- The species that constitute a mixed biofilm and the interactions between these microorganisms critically influence the development and shape of the community.
- In general, interspecies interactions involve communication, typically via quorum sensing, and metabolic cooperation or competition.

Schaechter,2004; Elias and Banin,2012

Biofilm

Multispecies/monospecies microbial community

- Interactions among different species within a biofilm can be antagonistic, such as competition over nutrients and growth inhibition, or synergistic.
- The latter can result in the development of several beneficial phenotypes.
- These include the promotion of biofilm formation by co-aggregation, metabolic cooperation where one species utilizes a metabolite produced by a neighboring species, and increased resistance to antibiotics or host immune responses compared to the mono-species biofilms.

Elias and Banin,2012

Biofilm
Multispecies microbial community

- In natural environments, the biofilm is almost invariably a multispecies microbial community harboring bacteria that:

1. Stay and leave with purpose,
2. Share their genetic material at high rates, and
3. Fill distinct niches within the biofilm.

- Thus, the natural biofilm is:

1. Less like a highly developed organism, and
2. More like a complex, highly differentiated, multicultural community much like our own city.

Watnick and Kolter,2000

Biofilm

Biofouling or biological fouling

Microbiological fouling/microfouling

- Biofouling or biological fouling: The damage caused to a surface by microorganisms attached to a surface.
- Biofouling can be either:

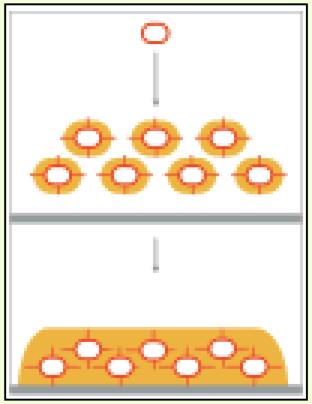
1. Micro (by bacteria, slimes, algae, and the like);
2. Macro (by clams, mussels,...) in nature.


Daniels and Selby,2007

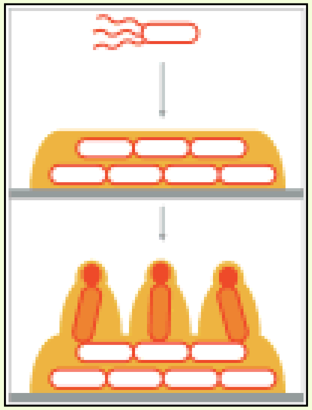
Two general models for biofilm development

Nonmotile and motile bacteria

- In nonmotile bacteria, changes in cell surface proteins, along with the production of EPS, play a critical role in the initiation of biofilm formation.

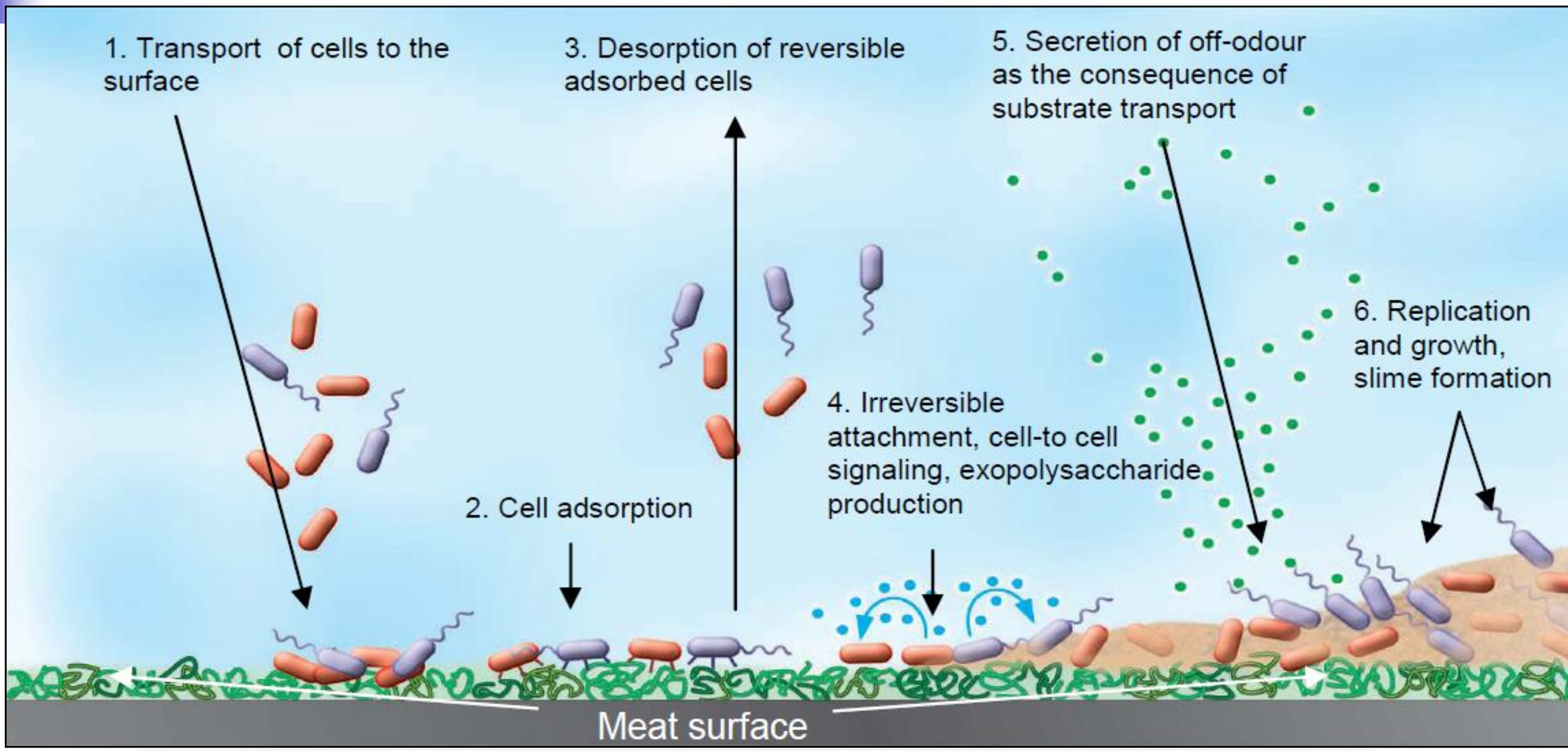


- For a number of motile organisms, the dominant role for flagella in initiation of biofilm formation is to provide motility as flagella-minus and paralyzed flagella mutants are comparably defective in biofilm formation.



Lemon *et al.*,2008

Biofilm development
Meat surface
A glance



Desorption is a phenomenon whereby a substance is released from or through a surface. The process is the opposite of sorption/adsorption (that is, either adsorption or absorption).

Marta *et al.*,2011

Steps in biofilm development

A glance of a developing *P. aeruginosa* biofilm

- Stage 1, initial attachment;
- Stage 2, irreversible attachment;
- Stage 3, maturation I;
- Stage 4, maturation II;
- Stage 5, The mature, fully functioning biofilm ready for dispersion.
- All photomicrographs are shown to same scale.

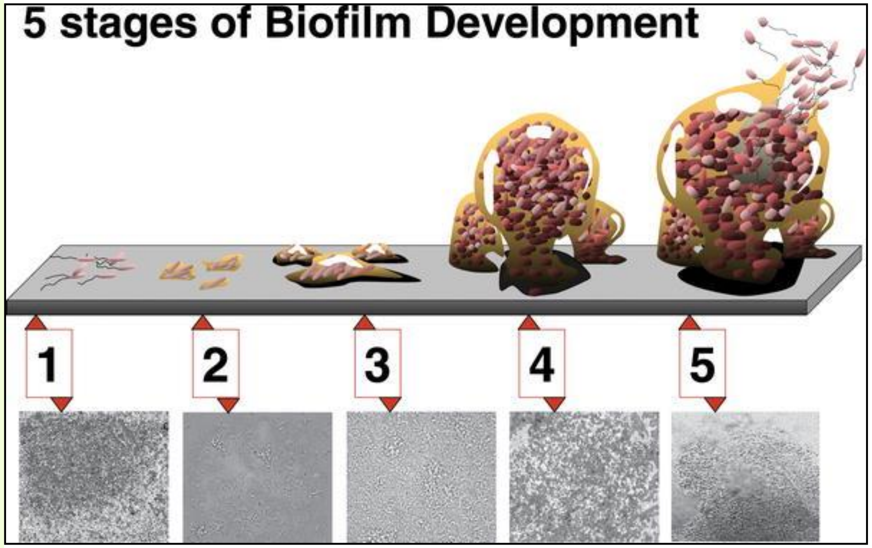
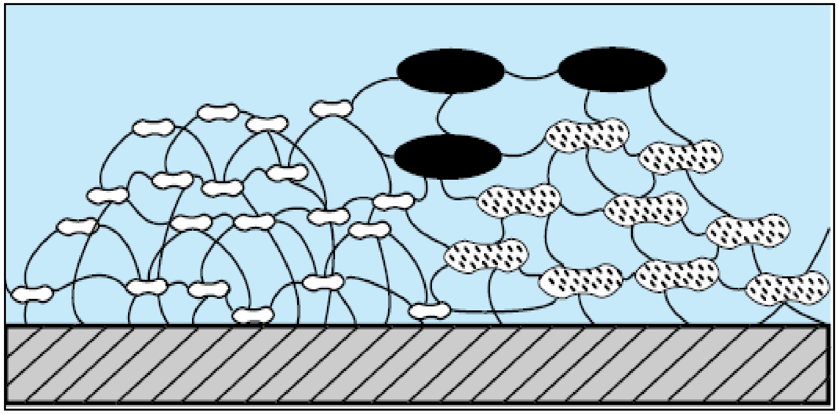


Image Credit: D. Davis

Monroe,2007

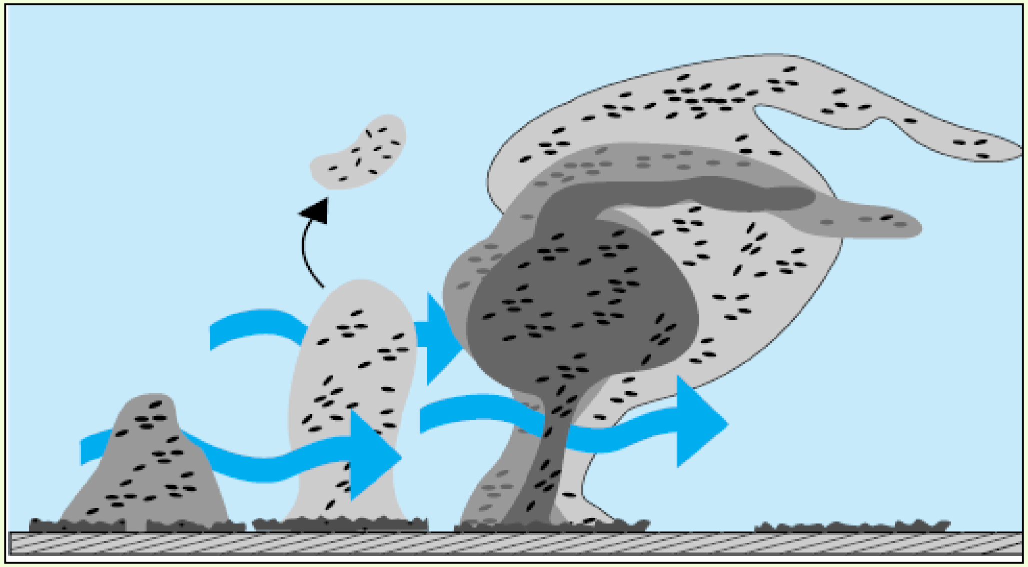
Steps in biofilm development
Pioneer cells

- A biofilm starts when a few pioneer cells use specialized chemical hooks to adhere to a surface.
- Bacteria in biofilms bind together in a sticky web of tangled polysaccharide fibers which anchor them to surfaces and to each other.



Steps in biofilm development

- Conceptual model of the architecture of a single-species biofilm based on direct observations using a confocal microscope (Costerton,1995).



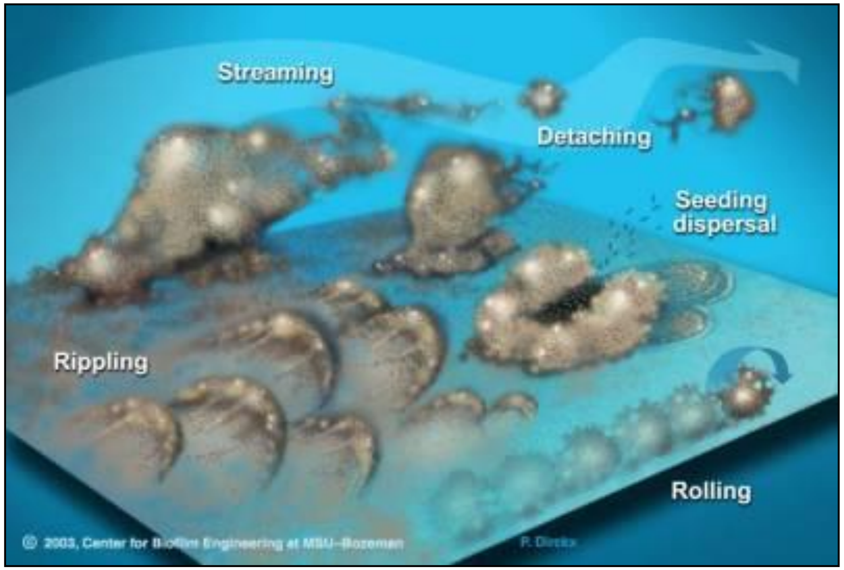
Steps in biofilm development

Biofilm migration

- Biofilm bacteria can move in numerous ways:

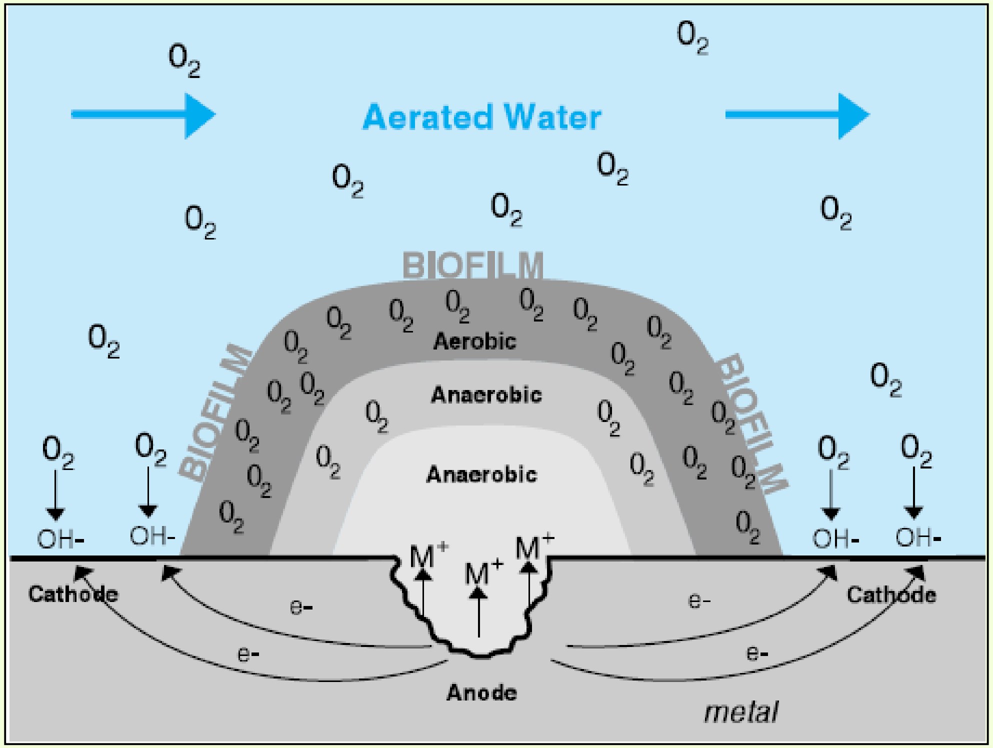
1. collectively, by rippling or rolling across the surface, or
2. by detaching in clumps.

- Individually, through a "swarming and seeding" dispersal.
- Source: Center for Biofilm Engineering, Montana State University-Bozeman



Oxygen is present at measurable concentrations mainly at the periphery of the biofilm

- Nonuniform colonization by bacteria results in differential aeration cells. This schematic shows pit initiation due to oxygen depletion under a biofilm (Borenstein,1994).



Biofilm development

The role of the three factors

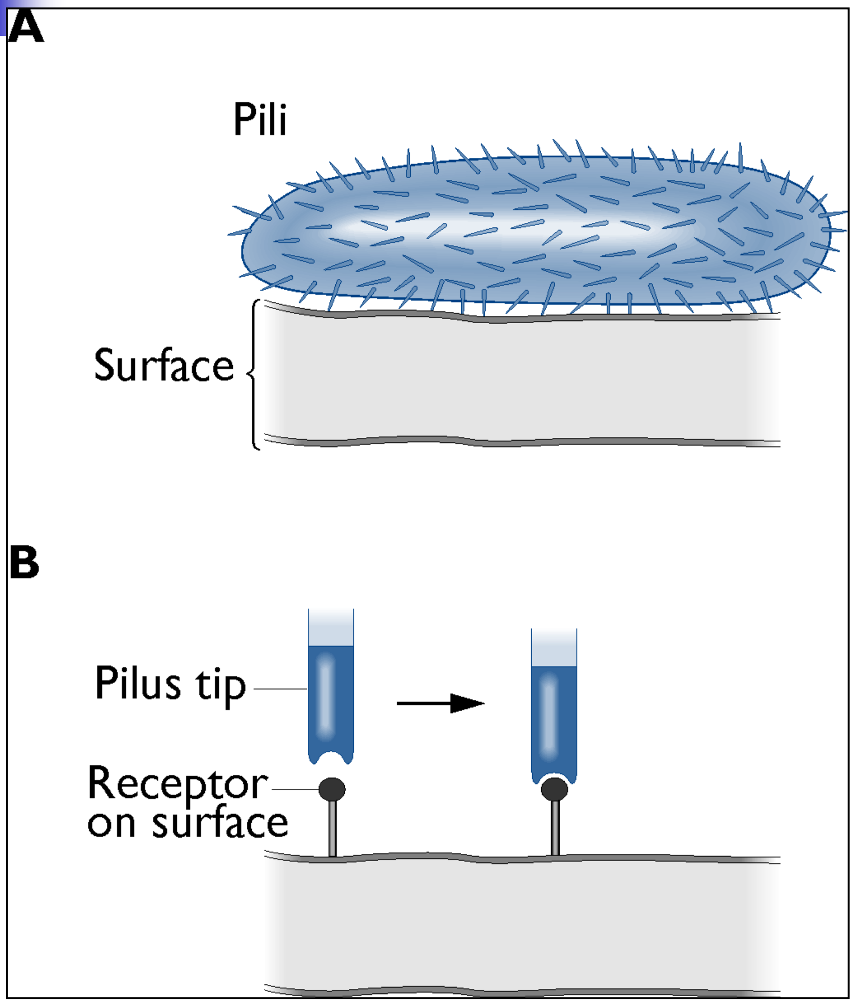
Pili, EPS, and LGT

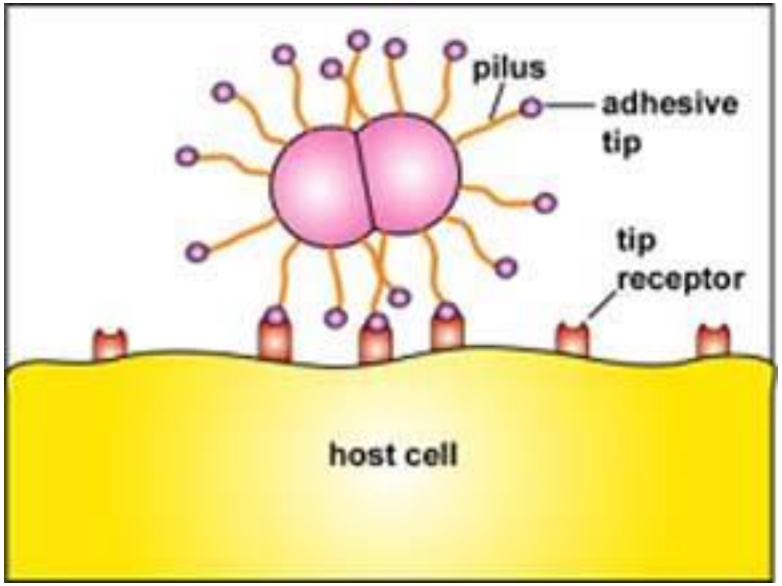
- Type IV pili, EPS production and lateral (or horizontal) gene transfer (LGT) are important for biofilm formation.

1. Most bacteria have type IV fimbriae (also called pili or its abbreviation “tfp”) for adhesion.
2. EPS not only determines the architecture, but also the strength and material properties of the biofilm.
3. Lateral gene transfer (LGT) is greatly facilitated in biofilms and leads to a more stable biofilm structure.

Attachment pili

Can be an important virulence factor a feature of the organism that enhances its ability to cause disease





Biofilm development

Type IV pili/fimbriae (“tfp”)

Twitching motility and Biofilm formation

- The Type IV pili are architectural marvels of biology.
- Type IV pili are remarkable multifunctional organelles expressed by diverse pathogenic bacteria.
- Twitching motility (surface motility) via type IV pili has been observed in a number of gram-negative bacteria.
- Type IV pili are essential for host colonization and virulence for many Gram negative bacteria, and may also play a role in pathogenesis for some Gram positive bacteria.

Ramey *et al.*,2008; Wikipedia,2010

Biofilm development

Type IV pili/fimbriae (“tfp”)

Type IV pili-related functions

- Type IV pili or fimbriae are non-flagellar, filamentous surface appendages that are associated with a number of biological activities in bacteria.
- These processes include:

1. A form of surface translocation (motility) termed twitching motility;
2. Bacteriophage sensitivity;
3. Attachment to biotic (bacteria, plant, animal);
4. Abiotic surfaces;
5. Biofilm development;
6. Uptake of naked DNA by natural;
7. Transformation.

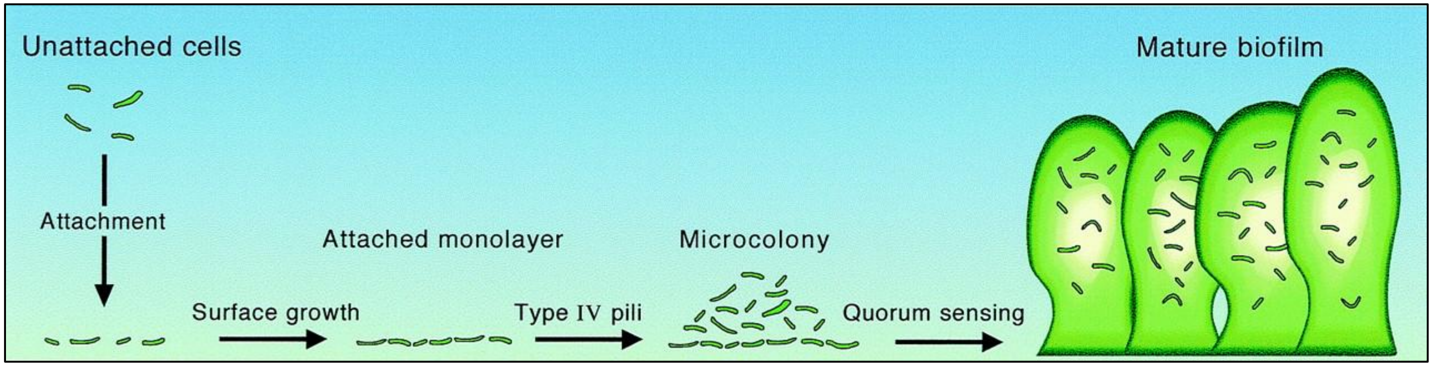
- Many of these biological functions are reliant on the ability of these structures to extend and retract.

Biofilm development
Pseudomonas aeruginosa

- Individuals in the layer then exhibit a surface motility called twitching.
- Twitching depends on type IV pili.
- As a result of twitching motility, small groups of *P. aeruginosa* called microcolonies form.
- Microcolonies then differentiate to form a mature biofilm.
- Microcolonies in a mature biofilm have tower- and mushroom-shaped architectures.
- Water channels that allow the flow of nutrients into and waste products out of the biofilm innervate these structures.
- There is a significant physiological heterogeneity within biofilms.

Biofilm development
P. aeruginosa

- Diagram of the *P. aeruginosa* biofilm-maturation pathway.
- Unattached cells that approach a surface may attach.
- Attachment involves specific functions.
- Attached cells will proliferate on a surface and use specific functions to actively move into microcolonies.
- The high-density microcolonies differentiate into mature biofilms by a 3OC12-HSL-dependent mechanism.



Parsek and Greenberg, 2000

Biofilm development

R. solanacearum

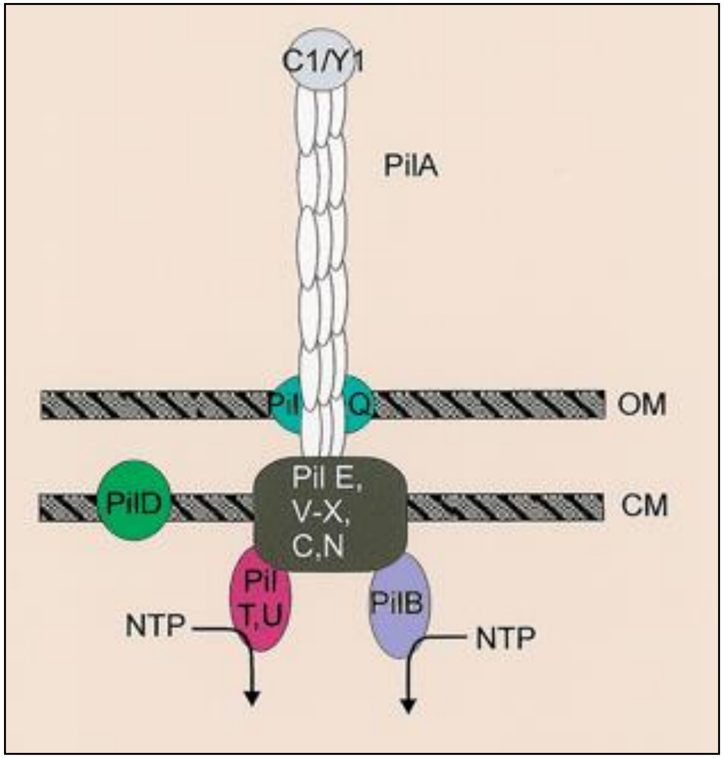
- Twitching motility (surface motility) is a form of bacterial translocation over firm surfaces that requires retractile type IV pili.
- Microscopic colonies of some *Ralstonia solanacearum* strains growing on the surface of a rich medium solidified with 1.6% agar appeared to exhibit twitching motility.
- Many of the genes required for production of functional type IV pili, and hence for twitching motility, are conserved among unrelated bacteria, and *pilD, pilQ* and *pilT* orthologues were identified in *R. solanacearum*.

Orthologues: Genes in different species that are homologous (similar) because they are derived from a common ancestral gene.

Pili structure

R. solanacearum

- Both *pilQ* and *pilT* mutants caused slower and less severe wilting on susceptible tomato plants to *R. solanacearum*.
- This is the first report of twitching motility by a phytopathogenic bacterium, and the first example where type IV pili appear to contribute significantly to plant pathogenesis.



Type I and type IV pili
Twitching motility and Biofilm formation
Xylella fastidiosa

- Particularly interesting is the fact that *X. fastidiosa* is the only bacterial species, to our knowledge, that possesses both types of pili at the same cell pole:

1. Type IV pili (1.0 to 5.8 μm in length), and
2. Type I pili (0.4 to 1.0 μm in length).

- This dual pilus configuration may confer advantages related to cell motility and biofilm development within the confines of xylem elements.
- Mutations in Type I and Type IV Pilus biosynthetic genes affect twitching motility rates in *Xylella fastidiosa*.
- The putative type IV pilus protein PilY1 is likely important for attachment to surfaces.

The T3SS machinery is absent in *X. fastidiosa*.

Fuente *et al.*,2007

Type I and type IV pili

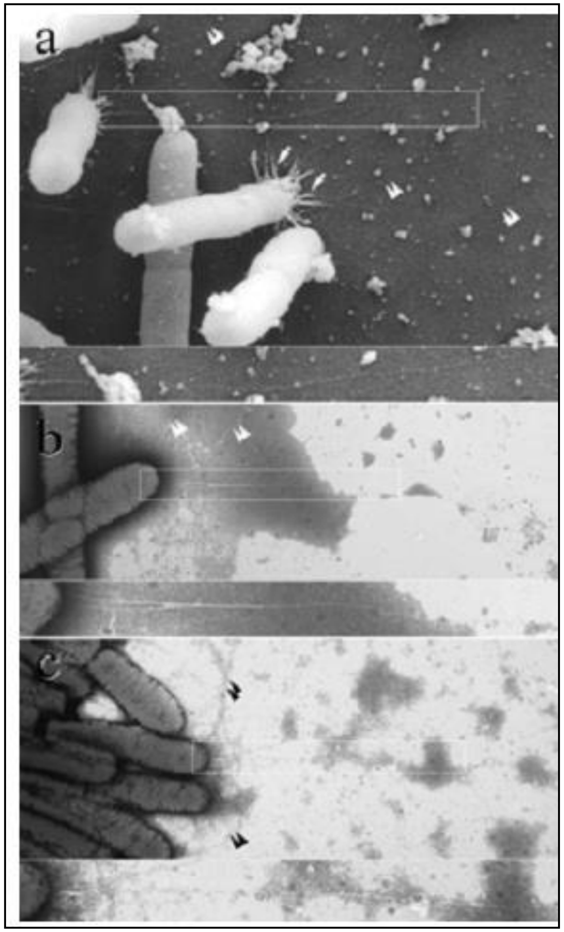
Twitching motility and Biofilm formation

Xylella fastidiosa

a) Wild-type cells depicting an abundance of short type I pili at the cell pole in contact with the substratum and fewer long type IV pili.

b, c) Transmission electron microscopy micrographs of *fimA* (A type I pilus mutant (b) and *pilY1* (A type IV pilus mutant cells (c) negatively stained with phosphotungstic acid.

- Only type IV pili are present on the fimA (b) mutant cells, whereas both pilus types are present on the pilY1 (c) mutant cells.



Fuente *et al.*,2007

Range of composition of biofilm matrices

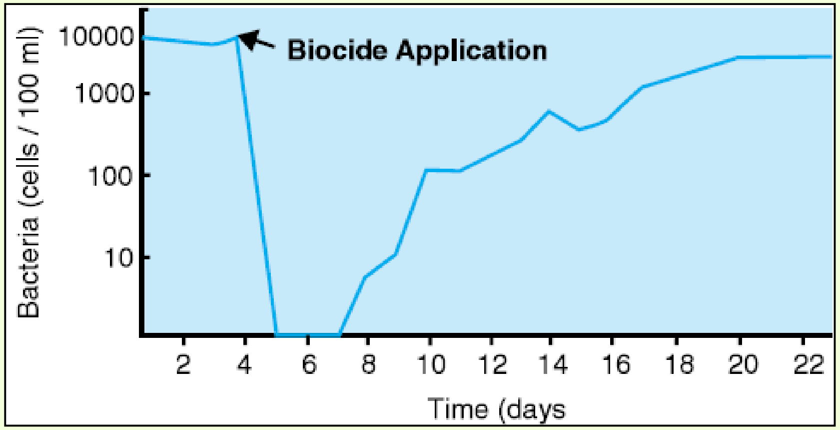
Component	% of matrix
Water	Up to 97%
Microbial cells	2-5% (Many species)
Polysaccharides (homo-and heteropolysaccharides)	1-2% (Neutral and polyanionic)
Proteins (extracellular and resulting from lysis)	<1-2% (Many, including enzymes)
DNA and RNA	<1-2% (From lysed cells)
Ions	? (Bound and free)

Sutherland,2001

Biocide application

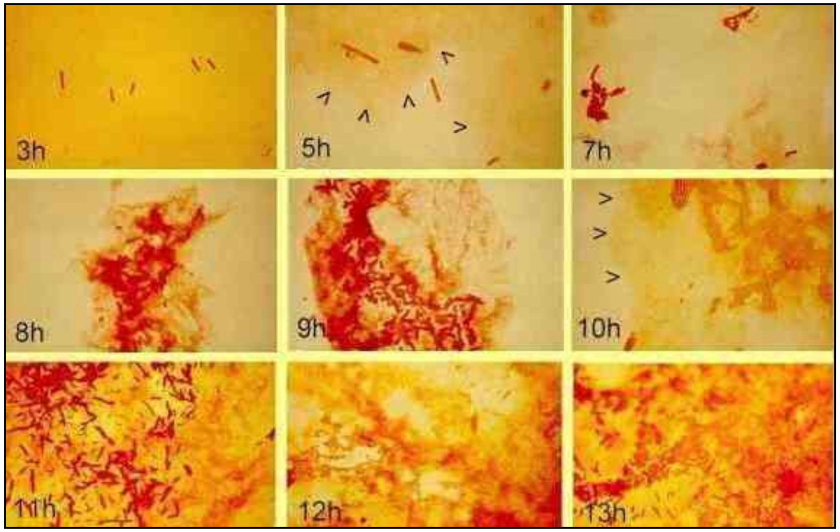
Removal and recovery of biofilms

- Reducing levels of nutrients in water can help reduce biofilm growth.
- It is common to observe a rapid regrowth of biofilm immediately biocide application.
- Incomplete removal of the biofilm will allow it to quickly return to its equilibrium state.
- Example of sanitization followed by biofilm recovery.
- Bacteria count samples were taken on a daily basis.



Microscopic examination
Biofilm congo red staining method

- *Pseudomonas* strain S61 was allowed to develop as a biofilm on glass slides immersed in nutrient medium containing 1% glucose.
- Development of the biofilm was followed by a specific staining technique (Congo red) in which:
  - The bacterial cells stain dark red and
  - The exopolysaccharide stains orange-pink.
- Attached cells can be seen at 3 hours.
- They then divide and form microcolonies.
- After 5 hours the development of exopolysaccharide is clearly seen (arrowheads) and it increases as the microcolonies increase in size.



Biofilm formation

Biofilm crystal violet staining method

- Biofilm formation was studied using 96-well microtiter polystyrene plates (O'Toole,2011).
- The overnight cultures were diluted with LB to the same starting optical density. The wells of the microtiter plate were filled with 200µl of rich media (i.e. LB) and then 20µl of diluted overnight culture was added.
- After incubating the plate at 28°C for 24 hours, the culture medium was removed, the wells were washed once gently with distilled water and 300 µl of 1% crystal violet solution was added to each well.
- The plate was stained for 30 minutes at room temperature, the stain solution was removed and the wells were washed twice with distilled water.
- Plate was allowed to dry and 300 µl of 70% ethanol solution was added to each well. The dye was allowed to solubilize by incubating the plate for 30 minutes and pipetting the content of the wells. The results were obtained by measuring OD_{600}.

Lagonenko *et al.*,2013

Biofilm formation

Biofilm crystal violet staining method

Quantifying the Biofilm

1. Add 125 µL of 30% acetic acid in water to each well of the microtiter plate to solubilize the crystal violet (CV).
2. Incubate the microtiter plate at room temperature for 10-15 min.
3. Transfer 125 µL of the solubilized CV to a new flat bottomed microtiter dish.
4. Quantify absorbance in a plate reader at 550 nm using 30% acetic acid in water as the blank.

O'Toole,2011

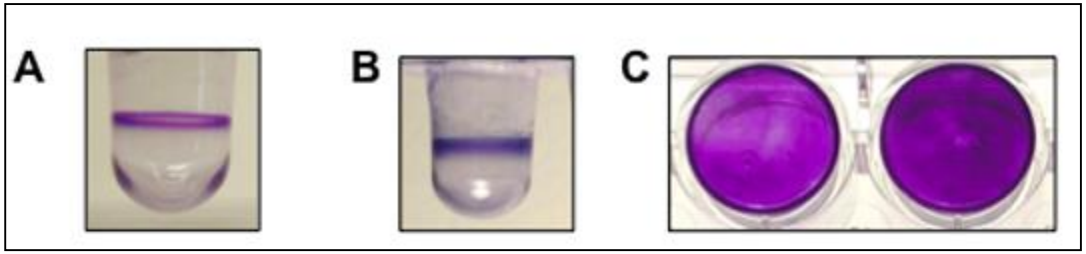
Biofilm formation

Biofilm crystal violet staining method

Quantifying the Biofilm

A. A side view of the well with a biofilm of P. *aeruginosa* (8 hrs, 37°C).
B. A side view of the well with a biofilm of *P. fluorescens* (6 hrs, 30°C).
C. A top-down view of the biofilm formed by *S. aureus* in a flat-bottom microtiter plate (two wells, 24 hrs, 37°C).

- *P. aeruginosa* and *P. fluorescens* are both motile organisms and form a biofilm at the air-liquid interface. *S. aureus* is non-motile and forms a biofilm on the bottom of the well.



O'Toole,2011

Biofilm management

- The slow metabolism of biofilm cells also contributes to their resistance.
- The bacteria in the film are relatively quiescent (inactive) and divide only rarely.
- Antibiotics such as the penicillins, which need to be incorporated in the cell wall, are only effective against actively dividing cells.
- However, other antibiotics work just as well against quiescent cells, because they target basic cellular processes such as metabolism or protein or DNA synthesis.
- For reasons that are still being clarified, even these antibiotics are less effective against biofilms.

Monroe,2007

Biofilm management

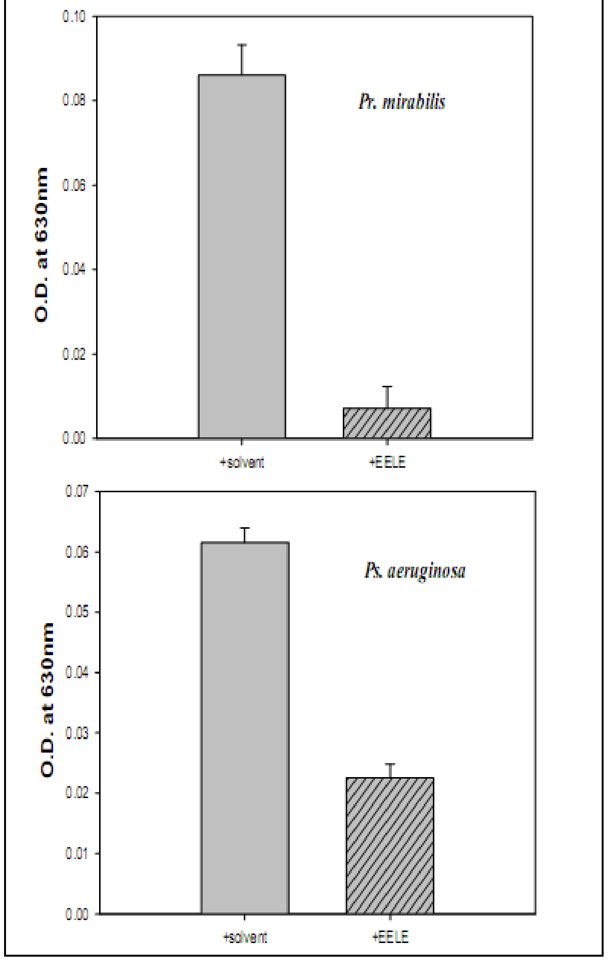
- It has been estimated that billions of dollars are spent every year worldwide to deal with damage to equipment, contaminations of products, energy losses, and infections in human beings resulted from microbial biofilms.
- Microorganisms compete, cooperate, and communicate with each other in multi-species biofilms.
- Understanding the mechanisms of multi-species biofilm formation will facilitate the development of methods for combating bacterial biofilms in clinical, environmental, industrial, and agricultural areas.

Yang *et al.*,2011

Biofilm management
Plant extracts treatments

- The assay was performed using the microtiter plate method in the presence of euphorbia (spurge) ethanolic latex extract, EELE (50 µlml^{-1}) for both *Proteus mirabilis* and *Ps. aeruginosa*.
- Wells containing an equal volume of 20% DMSO served as solvent control.

Fig. 5: Effect of EELE on biofilm formation of *Pr. mirabilis* and *Ps. aeruginosa*



Nashikkar *et al.*,2011

Biofilm management

Bacteriophage treatments

- Numerous bacteriophages of bacterial plant pathogens have been described in the past (Okabe and Goto,1963) including phages of *R. solanacearum* (Hayward,1964)and its banana-attacking strain (Buddenhagen,1960).
- At least one of these phages has been found to produce a bacteriolytic protein against *R. solanacearum*.

Biofilm management

Bacteriophage treatments

- EPS and biofilms render many bacteria resistant to treatment and environmental perturbations.
- One of the most exciting areas of research is the use of bacteriophage to penetrate biofilms and directly attack pathogens.
- Bacteriophages are natural predators of bacteria, and some carry genes that enable them to effectively depolymerize biofilms.

Biofilm management

Bacteriophage treatments

- An attractive way to understand the role of EPS in more depth and eventually increase disease resistance by reduced EPS production in plant pathogenic bacteria could be through the use of polysaccharide depolymerase enzymes.
- Hartung *et al.*,1986 described the isolation of a polysaccharide depolymerase gene from a bacteriophage of *E. amylovora*.
- The purified recombinant enzyme degraded amylovoran, the acidic component of the EPS abolished the virulence of *E. amylovora* in bioassays, and inhibited bacterial cell growth.

Biofilm management

Bacteriophage treatments

- These observations indicate that correct expression of the gene in plants may be useful for testing this approach to control bacterial diseases.
- These bacteriophages apparently use additional ways to facilitate the infection of their host (bacteria), which includes a lysozyme and a holin that may form a pore to support cell lysis by the lysozyme.

Holins (from making holes) are proteins act by permeabilizing the host cell cytoplasmic membrane.

Salicylic acid

Different functions of salicylic acid on biofilm formation by plant pathogenic bacteria

- Salicylic acid (SA) is an important plant hormone whose concentration in plants is regulated by abiotic and biotic factors.
- It's a well-known fact that microbial infection induces accumulation of endogenous SA.
- As a result, the systemic acquired resistance occurs.
- We suggested that SA not only triggers plant defense responses but also directly affects production of virulence factors by plant pathogenic bacteria.
- SA is as an antimicrobial agent that directly affects cells of plant pathogenic bacteria.

Lagonenko *et al.*,2013

Salicylic acid

Inhibitory or promoting effects of SA on biofilm formation and bacterial motility

- Salicylic acid (SA), a plant-produced phenolic compound:

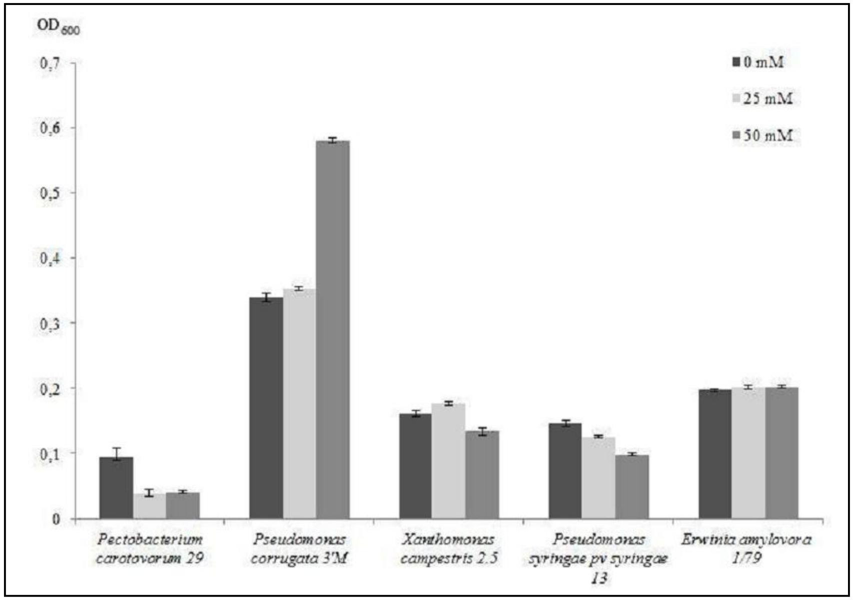
1. Inhibits biofilm formation, motility and N-Acyl homoserine lactone production by *Pectobacterium carotovorum* and *Pseudomonas syringae* pv. *syringae* at subinhibitory concentrations.
2. SA induces biofilm formation by *Pseudomonas corrugata* while inhibiting its motility.
3. SA had no effect on biofilm formation by *Xanthomonas campestris* pv. *campestris* and slightly increased its motility.
4. *Erwinia amylovora* was insensitive to SA treatment.

Lagonenko *et al.*,2013

Salicylic acid

1. Biofilm formation by different phytopathogenic bacteria in presence of SA

1. SA induces biofilm formation by *Pseudomonas corrugata*.
2. SA inhibits biofilm formation by *Pectobacterium carotovorum* and *Pseudomonas syringae* pv. *syringae* at sub inhibitory concentrations.

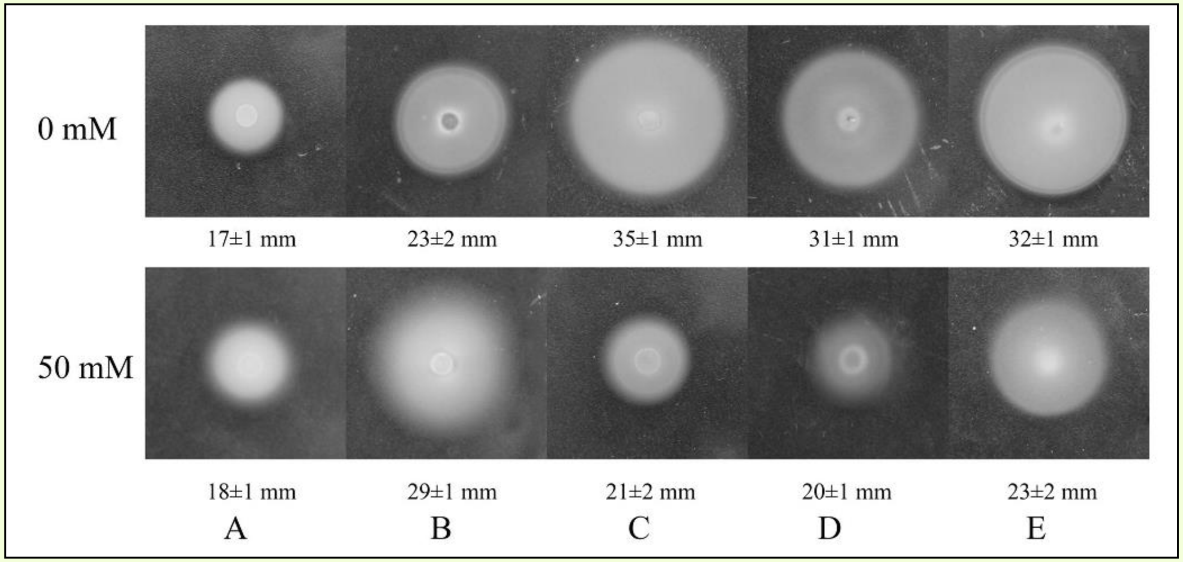


Lagonenko *et al.*,2013

Salicylic acid

2. Impact of SA on motility of different phytopathogenic bacteria in presence of SA

- A- *Erwinia amylovora* 1/79; B- *Xanthomonas campestris* pv. *campestris* 2.5; C- *Pseudomonas corrugata* 3′M; D- *Pseudomonas syringae* pv. *syringae* 13; E- *Pectobacterium carotovorum*.



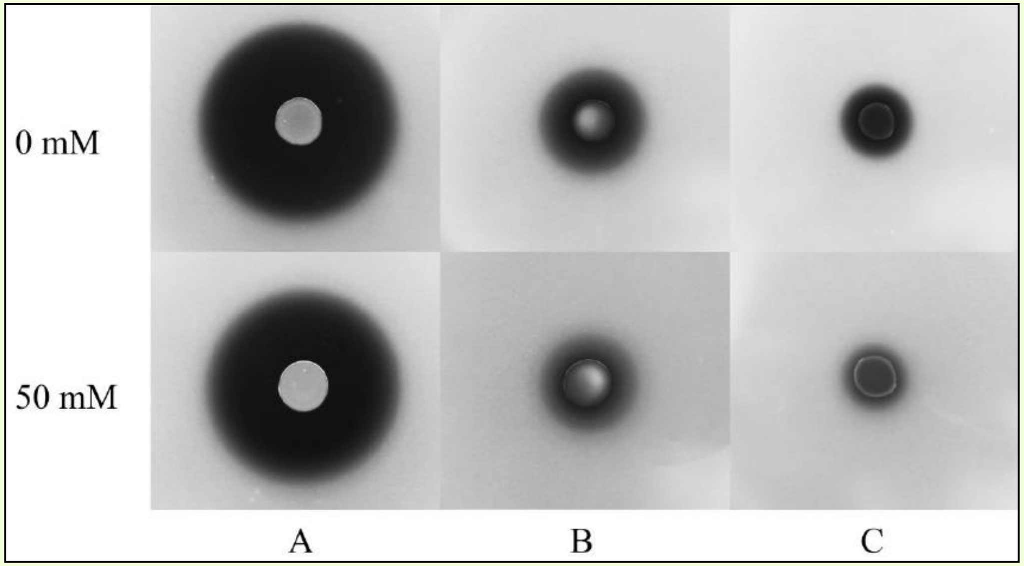
- SA inhibits *P. corrugata* (C), *Pectobacterium carotovorum* (D) and *P.syringae* pv. *syringae* (E) motility at sub inhibitory concentrations.

Lagonenko *et al.*,2013

Salicylic acid

3. Impact of SA on AHL production in different phytopathogenic bacteria

- SA inhibits N-Acyl homoserine lactone production by *Pseudomonas syringae* pv. *syringae* (B) and *Pectobacterium carotovorum* (C) and *Pseudomonas syringae* pv. *syringae* (C) at sub inhibitory concentrations. SA had no effect on AHL of *Pseudomonas corrugata* (A).



Violacein production was not induced by AHLs of *Xanthomonas campestris* pv. *campestris* 2.5 and *Erwinia amylovora* 1/79.

Lagonenko *et al.*,2013

Biofilm management

The seaweed *Delisea pulchra*

Biosignal's anti-biofilm technology

- Biosignal's anti-biofilm technology is based on the discovery that the red alga *Delisea pulchra* produces natural furanones that can disable bacteria's ability to colonize.
- **About the Product**
- **Company:** (formerly) BioSignal, Inc.
  Product Phase: Under development.
  Product Type: Biofilm control technology.
  Patent Name: Association of antimicrobial compounds with surfaces and polymers.
  Patent Number: WO 2005/053684 A1.

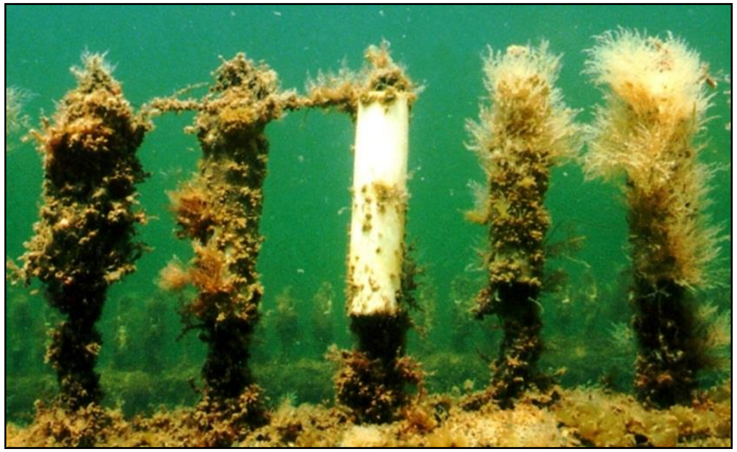
Marine algae, more commonly known as seaweeds. Furanones chemical structures similar to the *N*-acylhomoserine lactones may inhibit biofilm formation through interference with quorum sensing(QS signal-mimics).

Biofilm management

The seaweed(red alga) *Delisea pulchra*

Biosignal's anti-biofilm technology

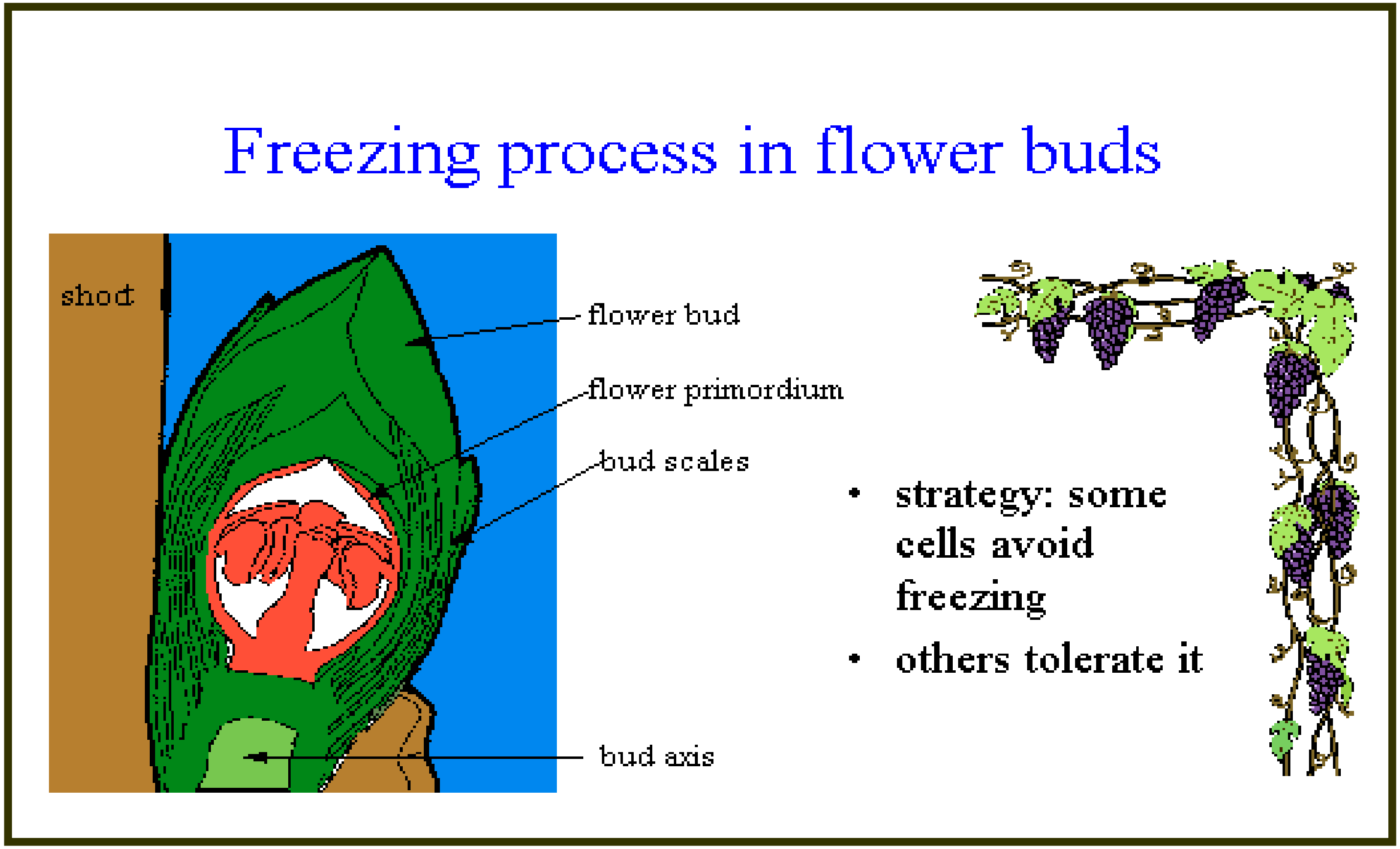
- Marine fouling is a major problem for waterborne craft around the world and results in significant additional fuel and maintenance costs for operators.
- BioSignal Ltd. is now testing and/or already applying synthetic furanones based on those produced by *Delisea pulchra* in a variety of applications.



Delisea pulchra protects itself from bacterial infection by exuding compounds that inhibit biofilms (middle image).

Fouling is the accumulation of unwanted material on solid surfaces to the detriment of function.

Ice nucleation bacteria
Freezing process in flower buds



Ice nucleation-active bacteria
INA$^+$ bacteria

1. *Pseudomonas syringae* pv. *syringae*
2. *Pseudomonas syringae* pv. *coronafaciens*
3. *Pseudomonas syringae* pv. *pisi*
4. *Pseudomonas syringae* pv. *lachrymans*
5. *Pseudomonas savastanoi* pv. *glycinea*
6. *Pseudomonas viridiflava*
7. *Pseudomonas fluorescens*
8. *Pantoea ananatis*
9. *Pantoea agglomerans*
10. *Xanthomonas translucens*

Sea ice microbial ecology
Cold-adapted microorganisms

- Sea ice is a dynamic, porous matrix that harbors within its interior network of brine pores and channels an active and diverse community.
- The sympagic (ice-associated) community has multiple trophic levels including:
- photosynthetic bacteria and algae, chemoautotrophic bacteria and archaea, and heterotrophic bacteria, archaea, flagellates, fungi and small metazoans.

Ewert and Deming,2013

Sea ice microbial ecology

Microbiology of the sea ice

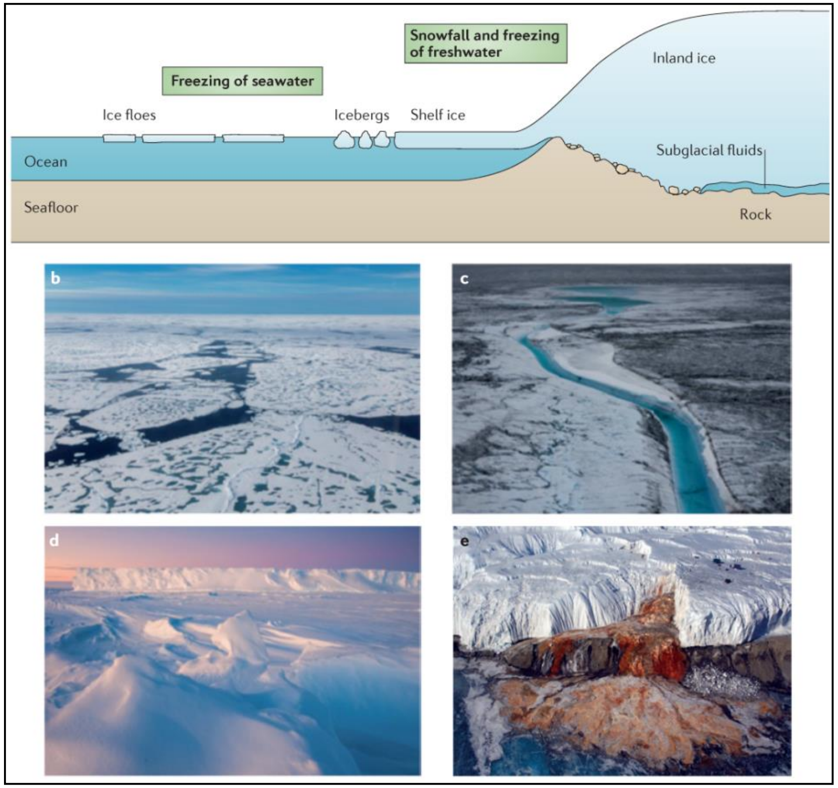
- The organisms living within the sea ice are consequently small (<1mm), and dominated by:
- bacteria,
- unicellular plants, and
- animals.
- Diatoms, a certain type of algae, are considered the most important primary producers inside the ice with more than 200 species occurring in Arctic sea ice.
- In addition, flagellates contribute substantially to biodiversity, but their species number is unknown.

Wikepedia,2017

Sea ice microbial ecology

Sea ice ecosystem

- Top: a schematic of different elements of the cryosphere.
- b: warm, summertime sea ice,
- c: the supraglacial environment, featuring a meltriver,
- d: cold winter sea ice,
- e: the subglacial environment, featuring the Blood Falls outflow from Taylor Glacier.



Boetius *et al.*,2015

Sea ice microbial ecology
Cold-adapted microorganisms

- *Melosira arctica* - key diatom species in the Arctic.

Legeżyńska,2009

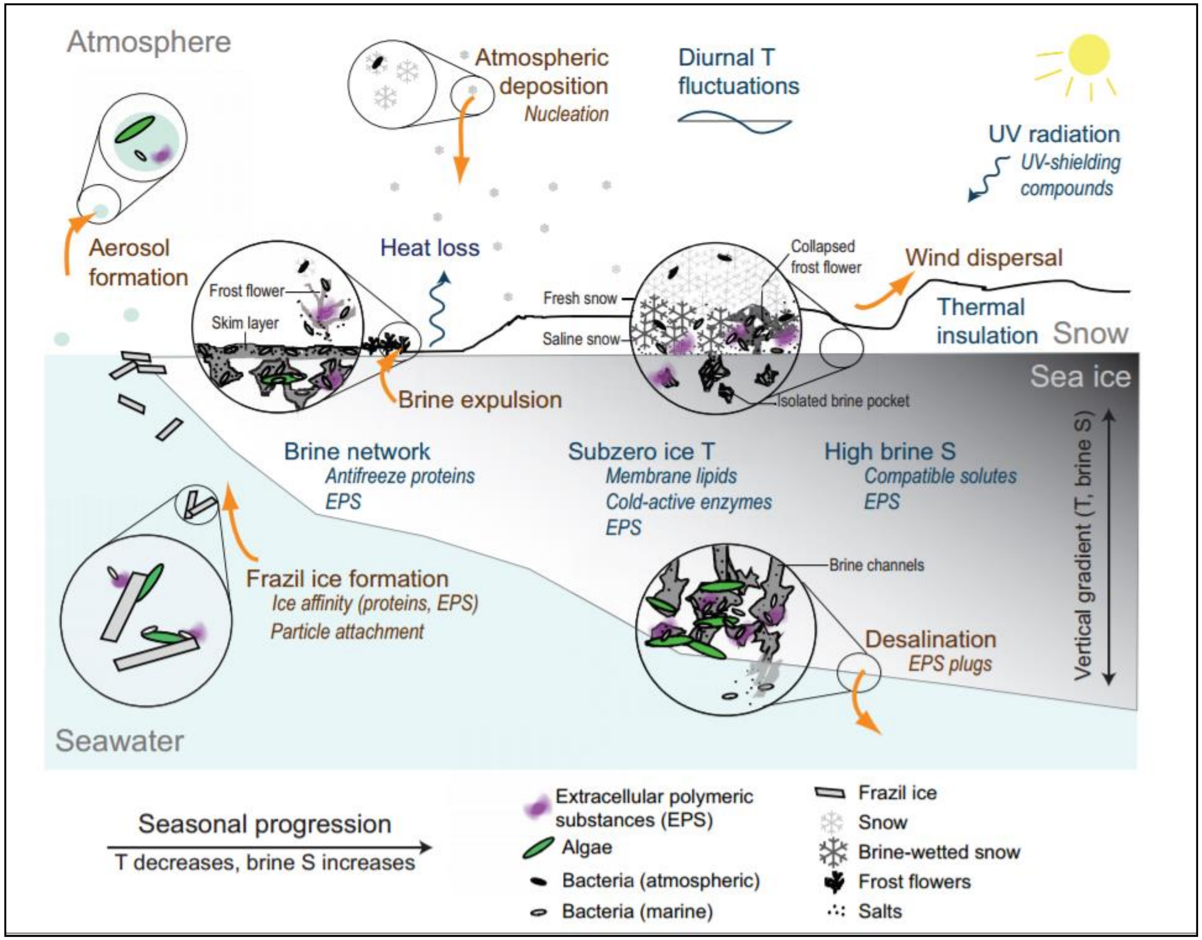
Sea ice microbial ecology
Cold-adapted microorganisms
Bacteria and algae

- Bacterial incorporation can be facilitated by the presence of algae, through bacterial association with algal cells or aggregated algal EPS, that are then concentrated by physical processes.
- Bacterial EPS also have the potential to play a role in the entrainment and retention of bacteria in ice either directly or by promoting attachment to algal cells or detrital particles amenable to physical entrainment.

Ewert and Deming,2013

Sea ice microbial ecology

Sea ice ecosystem

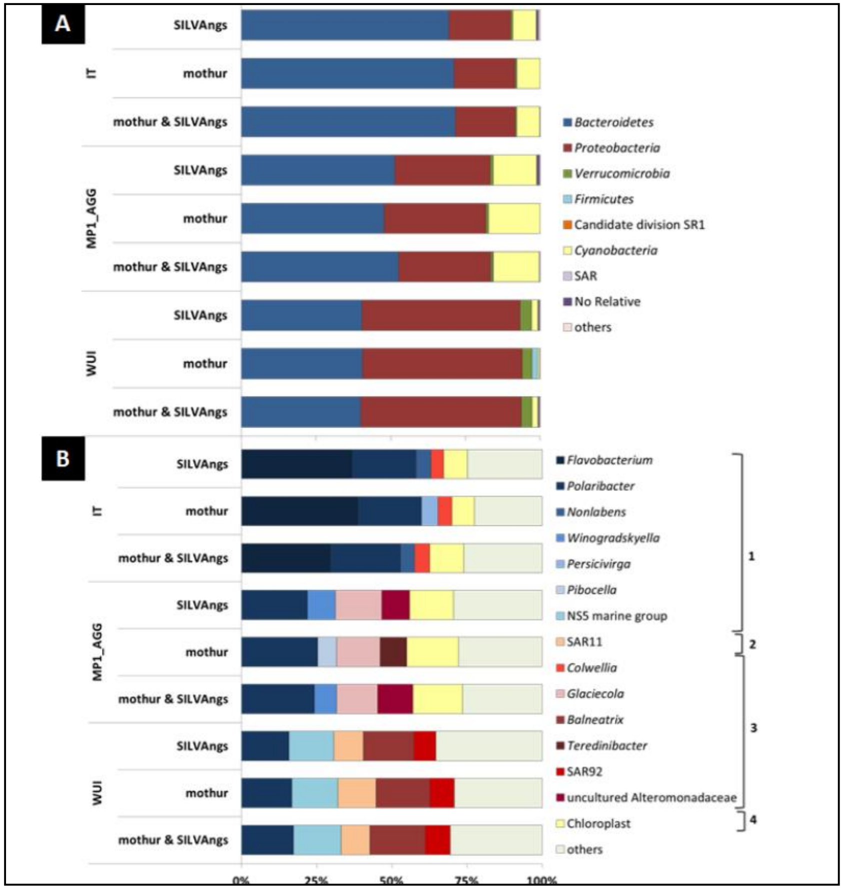


Ewert and Deming,2013

Sea ice microbial ecology
Cold-adapted microorganisms
Bacteria

- A: bacterial community composition at phylum level;
- B: bacterial community composition at genus level;

1. Flavobacteria;
2. Alphaproteobacteria;
3. Gammaproteobacteria;
4. Cyanobacteria.



Rapp,2014

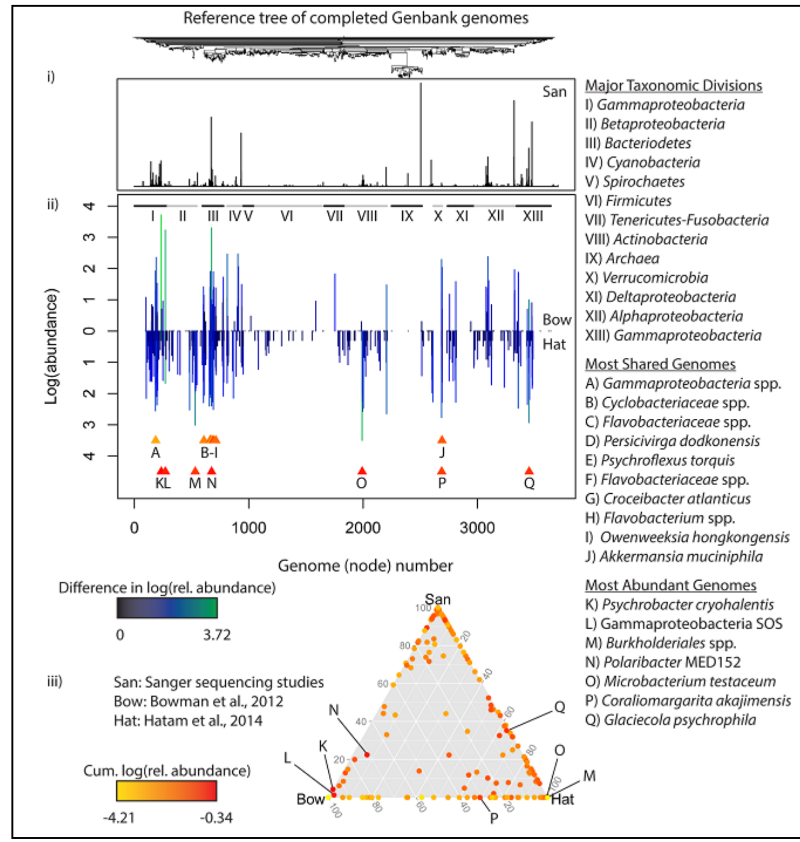
Sea ice microbial ecology

Community composition and structure in sea ice

i. Abundance of nodes in those studies that employed Sanger sequencing technology; abundance is scaled linearly on the y-axis.

ii. Abundance of nodes in the two available deep sequencing datasets.

iii. Ternary plot of node distribution in the three datasets.



Bowman,2015

Ice bacteria

Triggering of lightning

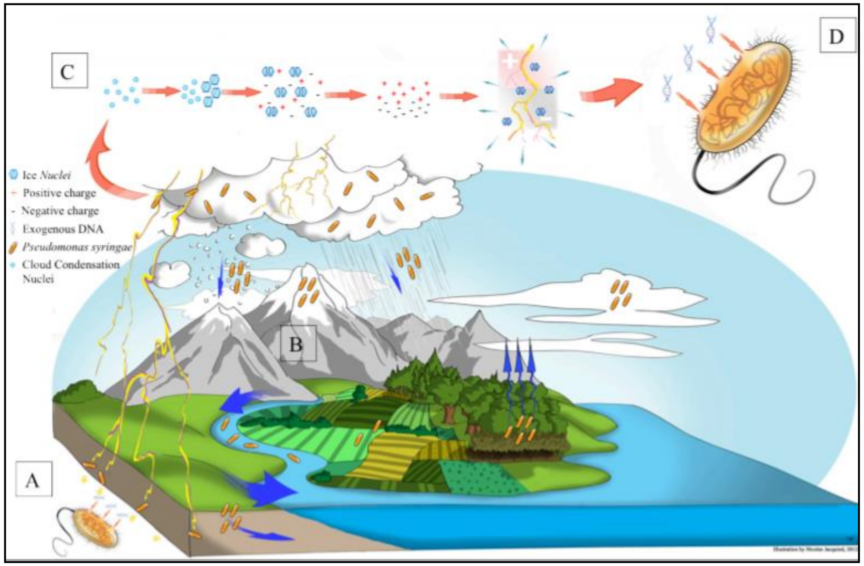
- Furthermore, some bacteria that possess ice nucleation abilities are involved in the triggering of lightning.
- The bacteria able to survive such a harsh physical and chemical environment may thus possess an important adaptive potential.
- For example, antibiotic resistant bacteria could reach clouds and be selected for either by passing through the harsh conditions of aerosolization or by the presence of chemical compound (Baker-Austin *et al.*,2006).
- Interestingly, despite leading to a decreased survival of bacteria, electric field pulses are also known to permeabilize bacterial membranes and thus to facilitate the penetration of naked DNA in the surviving cells and their acquiring of new properties (Demanèche *et al.*,2001c).

Laurine Blanchard,2013

Ice bacteria triggering of lightning

Electrotransformation has been proposed as a fourth potential transfer mechanism of genes in soil bacteria

- Electrotransformation mediated by lightning-related electrical phenomena may constitute an additional gene-transfer mechanism occurring in nature.
- The presence in clouds of bacteria such as *P. syringae* capable of forming ice nuclei that lead to precipitation, and that are likely to be involved in triggering lightning, led us to postulate that natural electrotransformation in clouds may contribute to the adaptive potential of these bacteria.



Laurine Blanchard,2013

Rain bacteria

Impact of lightning on rain bacteria

- Fluxes of bacteria from Earth surfaces (including soil, water and plants) to clouds result from aerosolization.
- The mean global emission of bacteria from terrestrial surfaces ranges from 140 to 380 CFU per square meter per second, only a small fraction of which reach the free atmosphere alive.
- So far, most studies have focused on the relationship between the presence of bacteria in clouds and geochemical processes; few have addressed the impact of cloud journeying on bacteria life cycles.
- Cloud bacteria are able to resist UV radiation, desiccation and high oxidative conditions or even to grow actively and to degrade chemical compounds in clouds.
- They are also submitted to electrical field pulses (fews $kV.cm^{-1}$) associated with lightning currents during lightning flashes.

Laurine Blanchard,2013

Rain bacteria

Rain collector

- Rain collector constituted a conic polyan canvas cover (about 1.875 m² in surface area), allowing rainwater to flow through a 0.5 mm nylon filter in a 150 mL Büshner funnel to be collected in a 2 L polyethylene sterile container.

Laurine Blanchard,2013

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