Nuevas tendencias en restauración de suelos degradados: tecnosuelos y fitorremediación (VITORIA-GASTEIZ, 2016)

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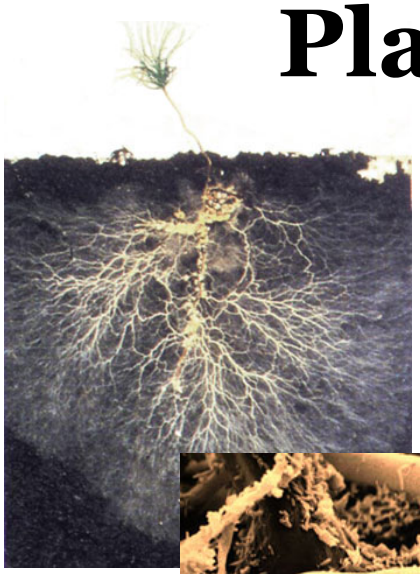
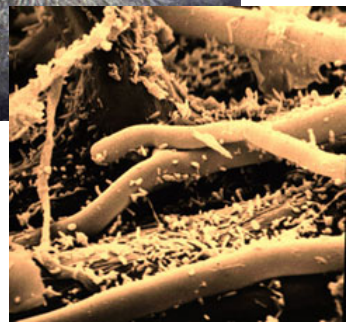
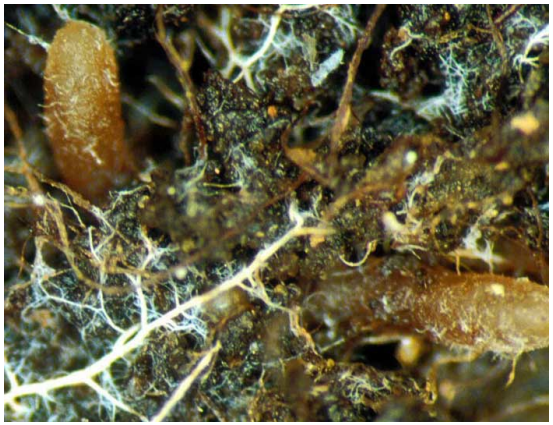
Facultad de Ciencia y Tecnología

Suelos-microorganismos y plantas: una relación a largo plazo

Plants-microbes in soil a "quid pro quo" relationship







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Dpto de Biología Vegetal y Ecología (UPV/EHU)

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Plants-microbes in soil a "*quid pro quo*" relationship

The basics: rhizoremediation, plants & microbiome

Understanding "phyto-spheres" in plants

Special status for some microorganisms: PGPBs, endophytes, symbionts

Essential issues in the *quid pro quo* relationship

New topics in plant microbione

Challenges and opportunities to improve rhizoremediation

The basics: plant microbiome

RHIZORMEDIATION, FUNCTIONAL TRAITS, PLANTS, MICROBIOME & SOIL

PLANT FUNCTIONAL TRAITS are key issues for effective PHYTOREMEDIATION

FUNCTIONAL TRAIT: a measurable plant property (BIOMAS, PRODUCTIVITY, PHYSIOLOGICAL STATUS, PHENOLOGY, HIPERACCUMULATION, DEGRADATION etc.) that correlate individual fitness (phenotype) to ECOLOGICAL PROCESSES AND SERVICES.

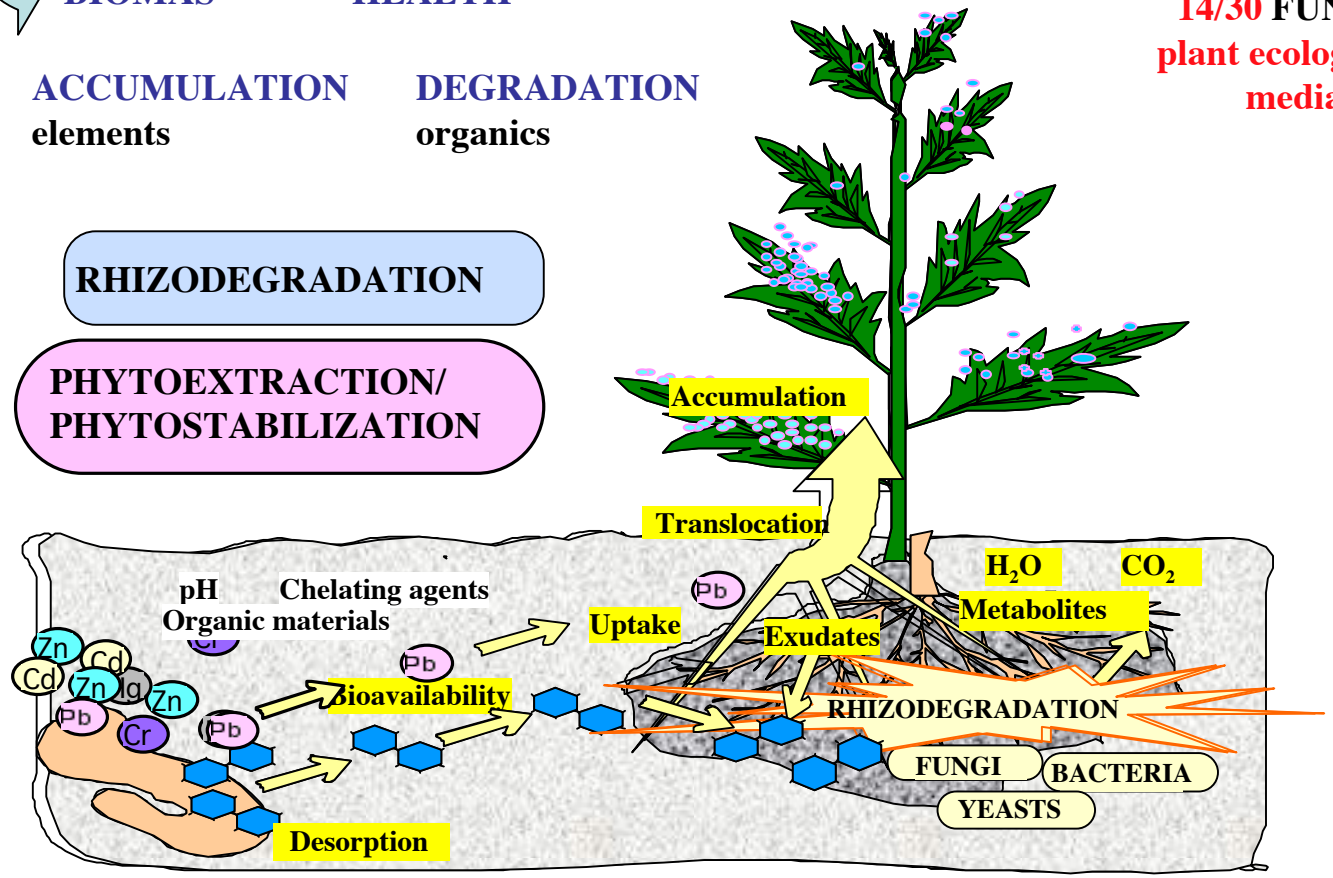
BIOMAS **HEALTH**

ACCUMULATION elements

DEGRADATION organics

RHIZODEGRADATION

PHYTOEXTRACTION/ PHYTOSTABILIZATION



14/30 FUNCTIONAL TRAITS used in plant ecological research are MICROBE mediated (Friesen et al., 2011)

NUTRIENT STATUS
ABIOTIC TOLERANCE
BIOTIC TOLERANCE
PLANT GROWTH
PLANT DEVELPMENT

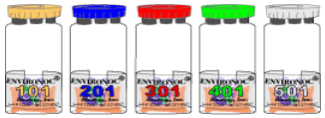
From "natural" strategies to "induced" strategies

RHIZOREMEDIATION

BIOSTIMULATION
BIOAUMENTATION

Bioaugmentation

Probiotic approach

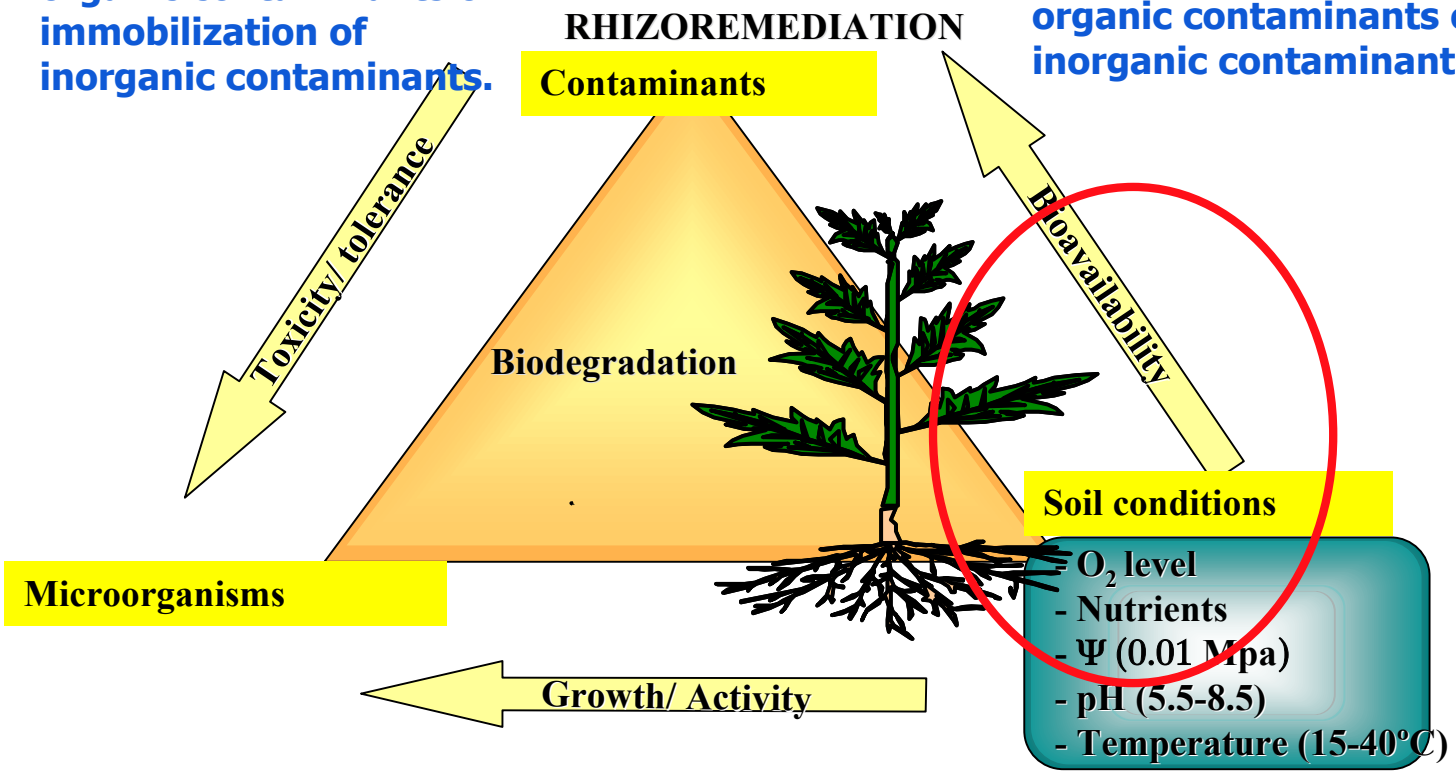


Microbes with special characteristics are added to enhance *in situ* biological degradation of organic contaminants or immobilization of inorganic contaminants.

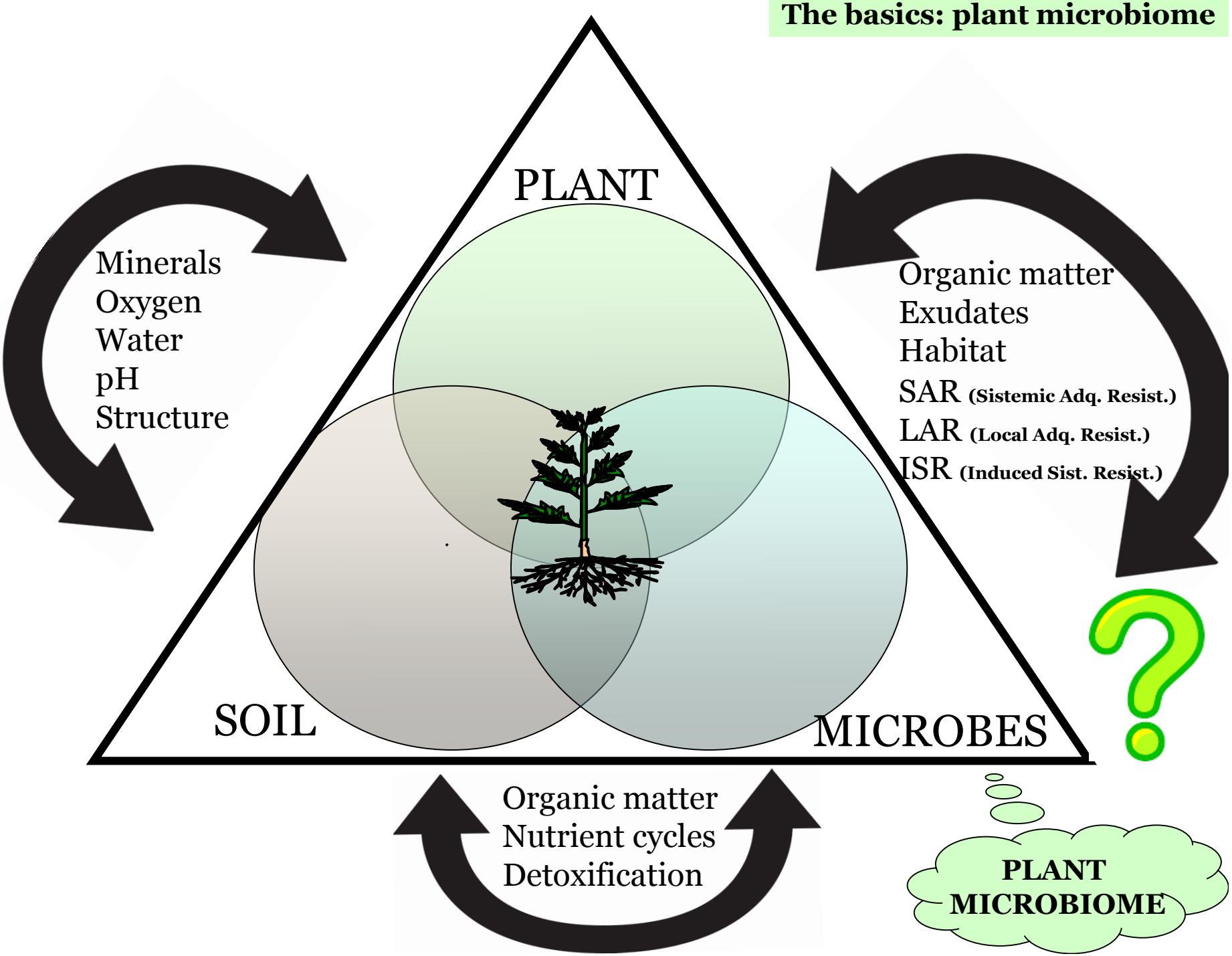
Biostimulation:

Prebiotic approach

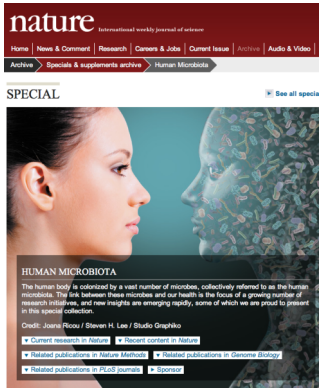
Nutrients, oxygen, or other amendments may be used to stimulate the activity of naturally ocurring microbes degradation of organic contaminants or immobilization of inorganic contaminants.



The basics: plant microbiome



Learning lessons from human microbiome



Joshua Lederberg (2001) "the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease"

-. Microbiome represent 90% of the number of cells of our body (Grice et al., 2012)

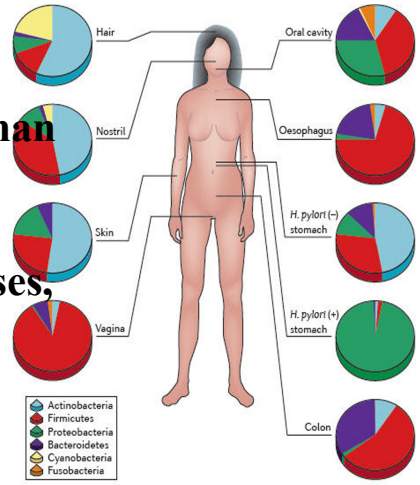
-. For every single human gene there are 100 genes within our microbiome (our second genome; microbiome is an extension of the host genome.

-. Human microbiome is composed of many microbiomes (gut, lung, skin, nose, etc.)

-. We have a "personal microbiome"

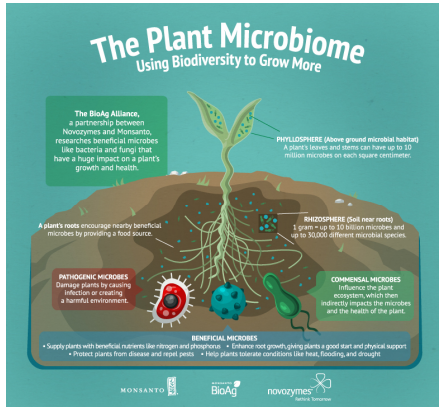
-. There is more differences in microbiome composition among the body sites than among individuals

-. Microbiome is involved in regulation of the immune system and genetic diseases, metabolic diseases, obesity, etc



ISR in plants

PLANT MICROBIOME what we start to know…



-. IT IS AN OLD RELATIONSHIP. Microbial associations with green algae were fundamental to the evolution of land plants about 700 million years ago.

-. PARALLEL ROLE to HM: second genome, individual microbiom, great differences in organ microbiomes, functions, etc.

-. ESSENTIAL ROLE: plant microbiome is a key determinant of plant health and productivity (plant fitness)

THE IMPORTANCE FOR A SESILE ORGANISMS COULD BE GREATER !!!

what we start to know… role in adaptation and acclimation: environmental fitness

-. Agricultural treatments or genetic modification of plants may have unplanned consequences for the microbiome.

-. It is important consider the plant microbiome when interpreting experimental data, especially field data.

-. The role of the microbiome in soil phytoremediation and its relationship to plant health, productivity, and biogeochemical cycles should be considered as much as the plant itself.

-. Manipulation of plant microbiome can reduce plant disease, improve agricultural production or soil remediation reducing chemical inputs and emissions of greenhouse gases.

ROLE OF MICROBIOME IS ESSETIAL FOR PHYTOMANAGEMENT!!!

Where is microbiota in plants?

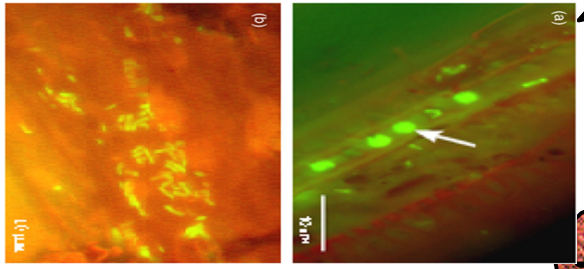
How close is the relationship?

pathogens vs PGPBs (Plant Growth Promoting Bacteria)

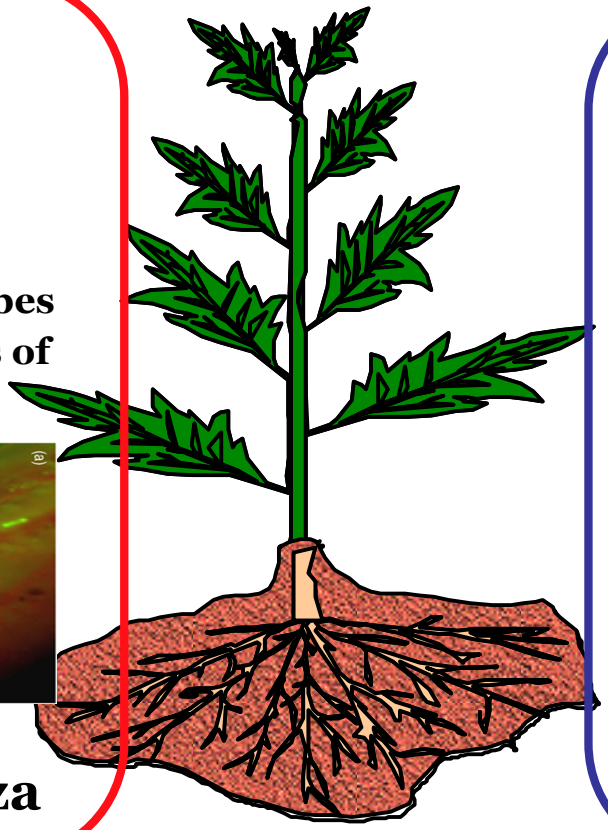
ENDOPHYTES

ENDOSPHERE

Endophytes: **microbes colonizing internal tissues of plants causing no damage**



nodule, mycorrhiza



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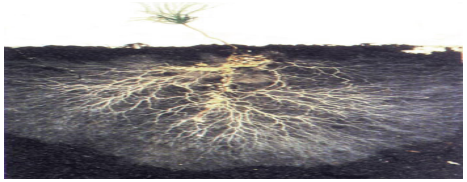


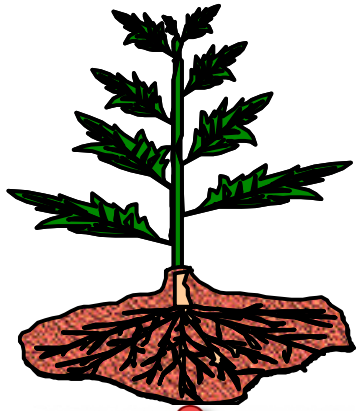
How we can identify and estimate plant microbiota?

Cultured microorganisms

Traditional tech.

Nutrient media
Growth conditions
Biolog (Funct. Diver)

2% of total bacteria in the environment



Uncultured microorganisms

New-omics tech.

PROCARYOTES- PCR 16S ribosomal RNA (taxonomic identification)

EUCARYOTES- PCR 18S ribosomal RNA

METAGENOMICS- gene global analysis

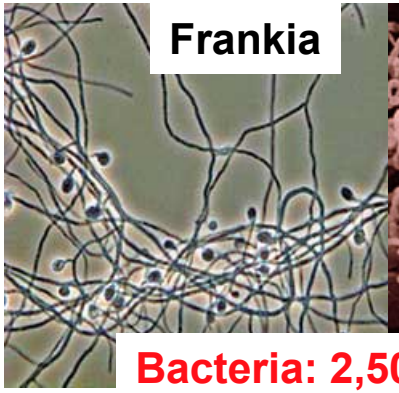
Ellis et al., 2013

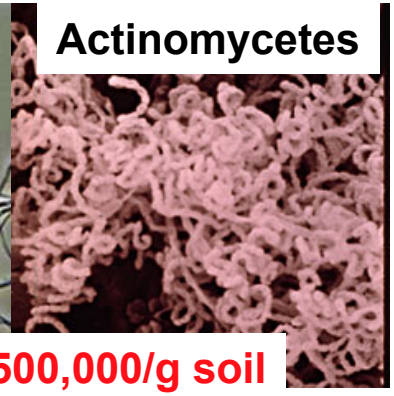
METAL CONTAMINATED SOIL

Cultured	2%	0,08%
Uncultured	no significant effect in genetic biodiversity	

Metal contamination may have NO significant effect on TOTAL GENETIC DIVERSITY as on PHYSIOLOGICAL STATUS

Identifying plant microbiota in the rhizosphere





Bacteria: 2,500,000/g soil

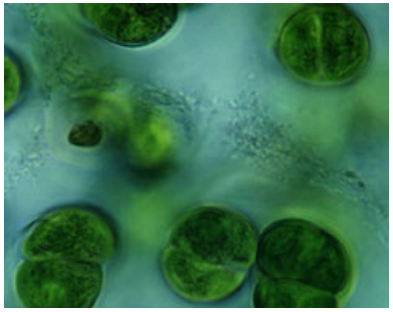
Fungi: 400,000/g soil

Mesofauna

Nematodes

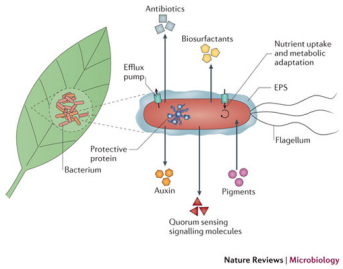
Macrofauna

Mites



Algae 50,000/g soil

Amoeba & protozoa
30,000/g soil



EPIPHYTES from PHYLLOSPHERE

How can a microorganism survive and benefit plants from surface ?

Phyllosphere a very hostile enviroment

Poor in nutrients
Strongly affected by leaf structure
Dynamic environment affected by biotic factors (light, UV, T^a, moisture, wind) in a very short period of time

Assimilate plant derived nitrogen (aa) and carbon compounds (G, F)
Hold a population of **Bacteria, yeast, fungi,(10^7 cells/cm^2 leaf)**

"quid pro quo"

Potential benefits:

Fix atmospheric nitrogen
Produce plant regulators
Photoprotection (UV)
Control of plant pathogens (phytoalexins, antibacterial or antifungal compounds

Not well understood!

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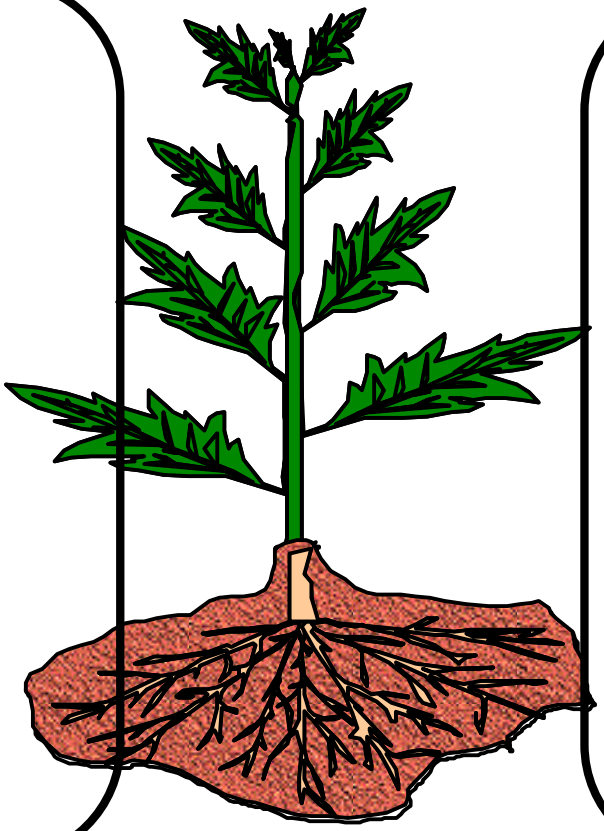


EPIPHYTES

PHYLLOSPHERE

Plant aerial surface of plants

RHIZOSPHERE

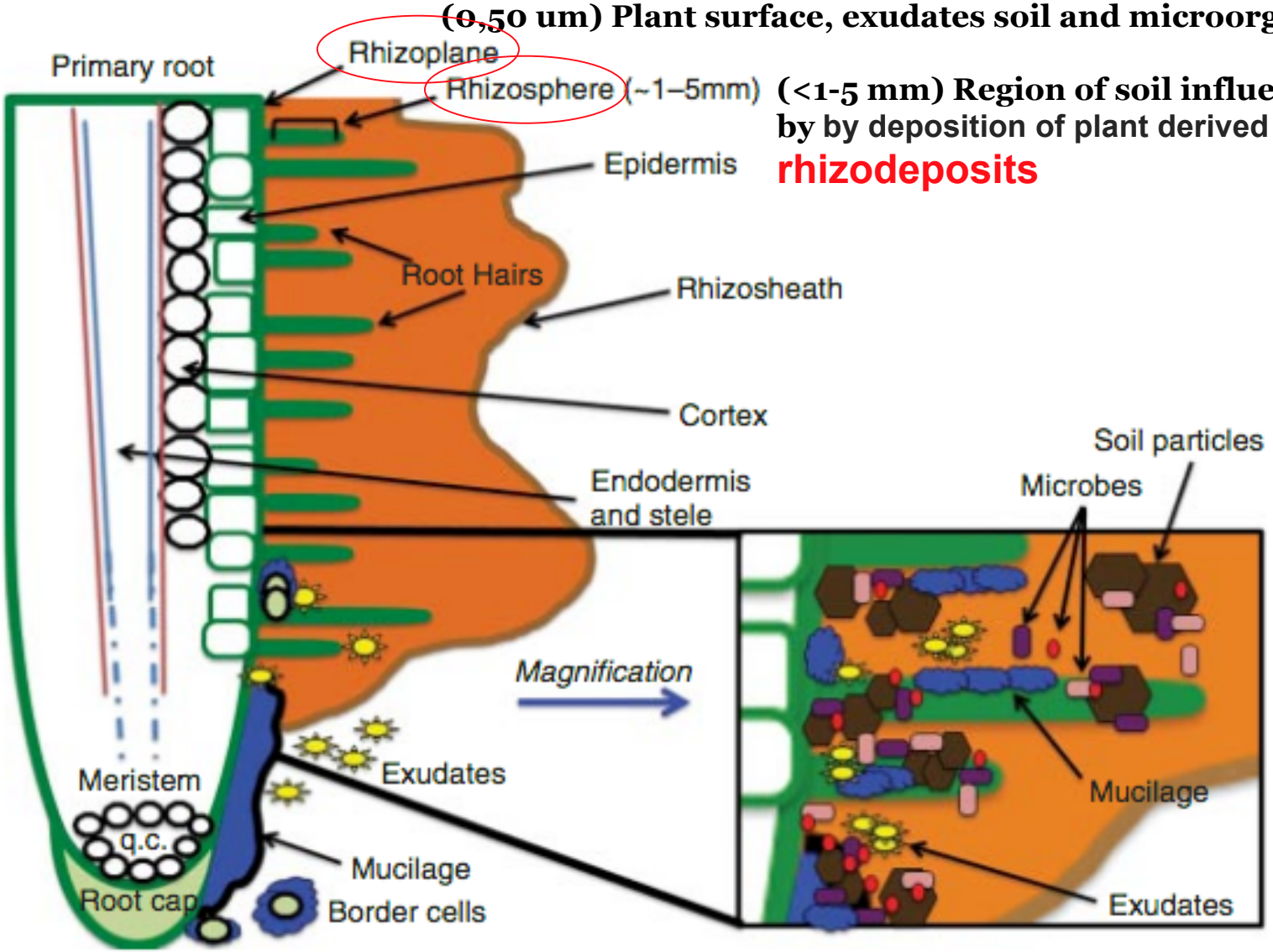
Region of soil influenced by by deposition of plant mucilage and root exudates and free cells.

RHIZOSPHERE

Functional zones of the roots for microorganisms?

(0,50 um) Plant surface, exudates soil and microorganisms

(<1-5 mm) Region of soil influenced by by deposition of plant derived rhizodeposits



After Angus & Hirsch, 2013

RHIZOSPHERE

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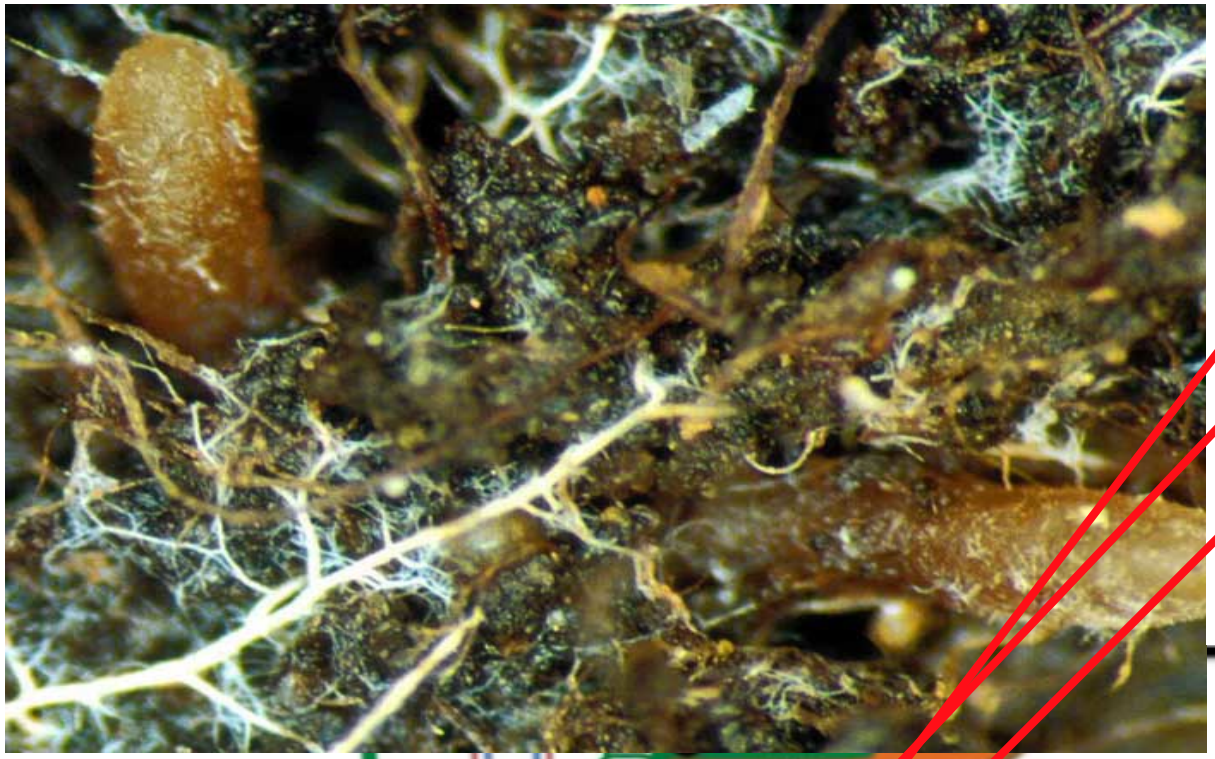
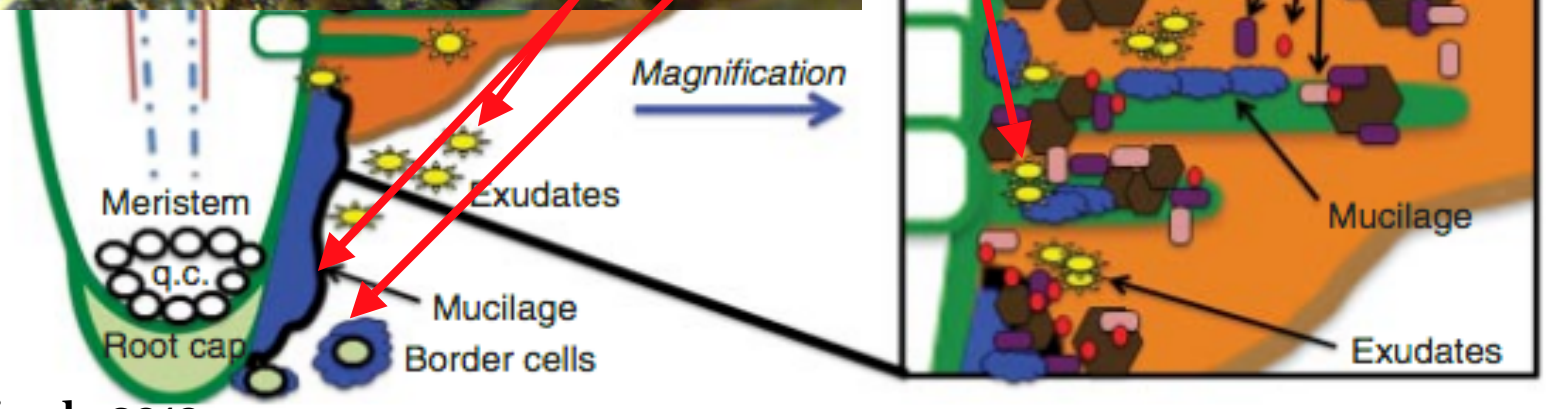


Root rhizodeposits
(exudates, secretions, lysates, cells, volatiles)

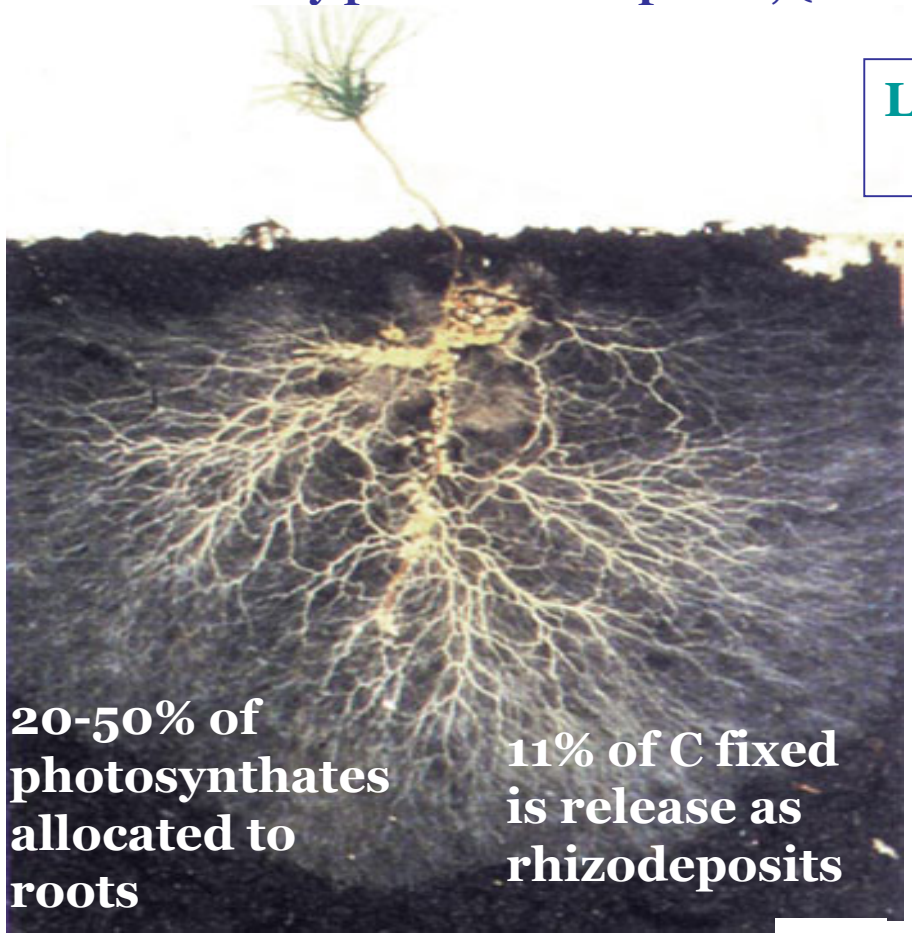
-. Soluble exudates
-. Mucilage 2-12%
-. (Biofilms)
-. Border/ Border like cells (10%)



After Angus & Hirsch, 2013

Root rhizodeposits: exudates

Microbiome composition of rhizosphere is driven (selective pressure) by plant rhizodeposits, (not only by soluble root exudates)



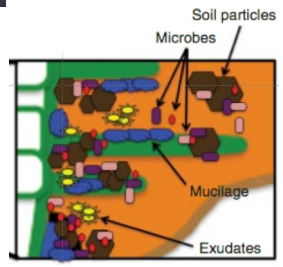
Low molecular vs High molecular weight

Composition

Sugars: monosac, polysac
Aa, proteins, Enzymes
Organic acids
Fatty acids
Sterols
Vitamins
Phenols
Nucleotides, DNA

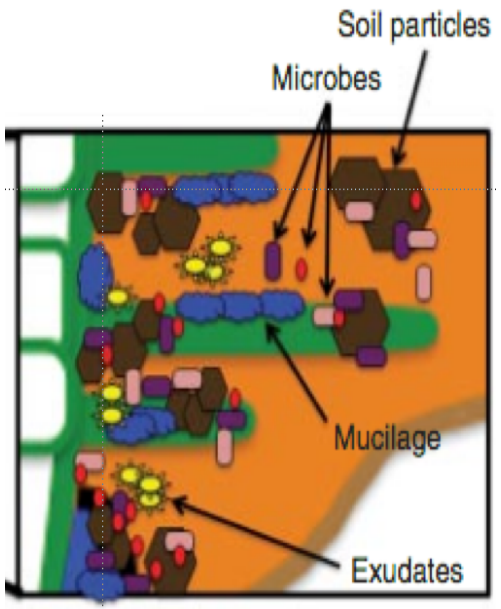
Others: inorganic ions
hormones
Phytoalexins
Phytotoxins
Allelochemicals
Phytosiderophores

Important points to be addressed:
-Composition
-Spatial/ temporal distribution

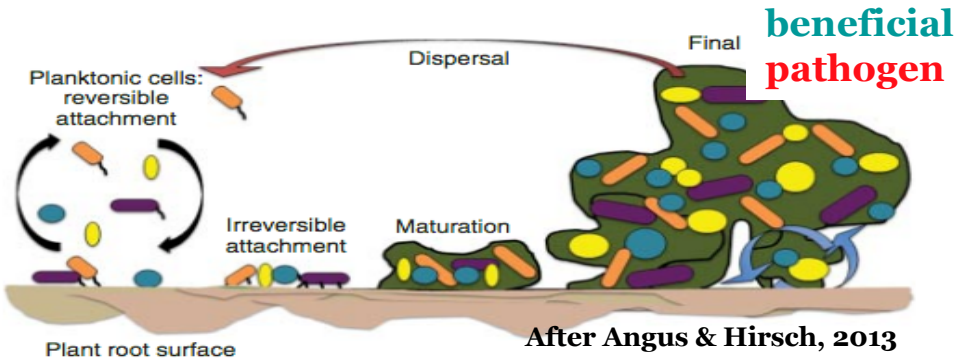


BIOFILMS

When bacteria "talk" each other form an "new peel" on root surface



Biofilm: is a community of associated microorganisms attracted to the same location (chemotaxis) and surrounded by a matrix of polymers (polysaccharides, proteins and DNA)



After Angus & Hirsch, 2013

Benefits for plants:

Protection and persistence fluctuating environments

Protection from abiotic stress: Tª, salt, desiccation nutrient deficiency, contaminants

Protection from biotic stress: pathogens (also form biofilms)

"quid pro quo"

nutrients
chemical signals

Successful biofilm formation rely on microbial communication

"Quorum sensing"

Percepción de quorum

When bacteria behave as a multicellular organism: QUORUM SENSING

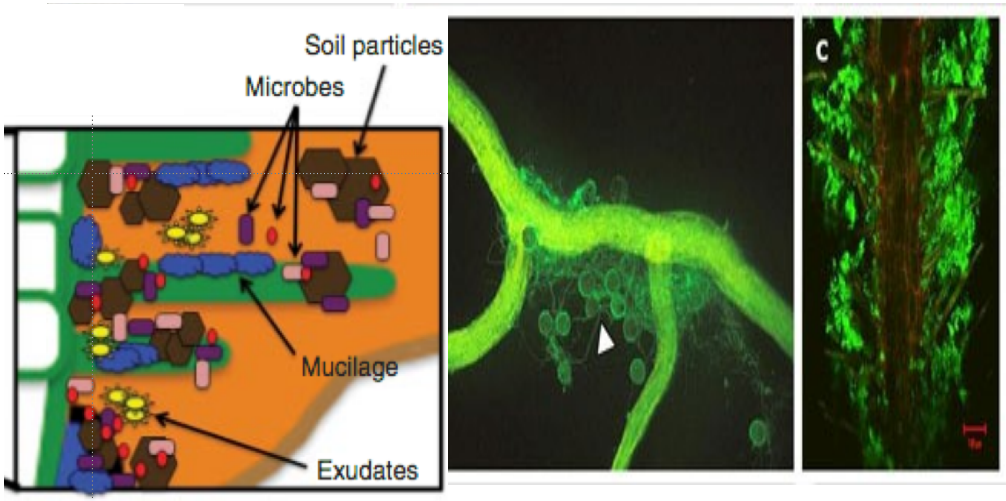
Quorum sensing: Chemical signalling allows individuals to act in coordination with the rest of the population and respond efficiently to changes or new conditions of the environment.

The process…

1.- Plants attract bacteria on the surface (exudates, nutrients, chemical signals)
2.- Bacteria constantly produce an specific chemical- autoinducer (acetylated homoserine)
3.- The signal accumulate to some extent (threshold level) due to the number of bacteria or similar signals produced by the plant, so "quorum sensing" is reached
4.- Induced change in gene expression of bacterial population acting in coordination to perform functions that single cells can not carry out

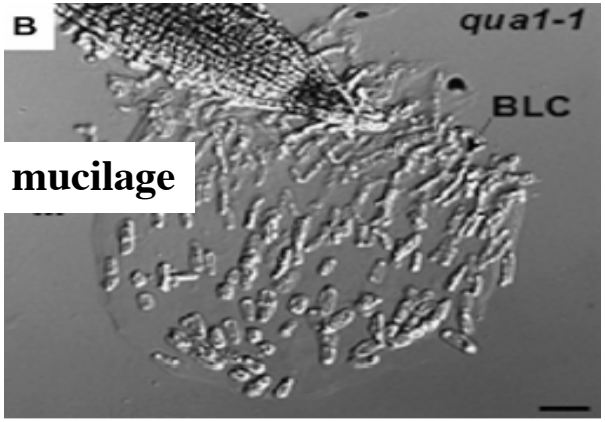
-. Production of exopolysacharide
-. Exoenzymes
-. Virulence
-. Plant protection
-. Nutrient mineralization
-. Desiccation protection
-. (In)solubilization of metals

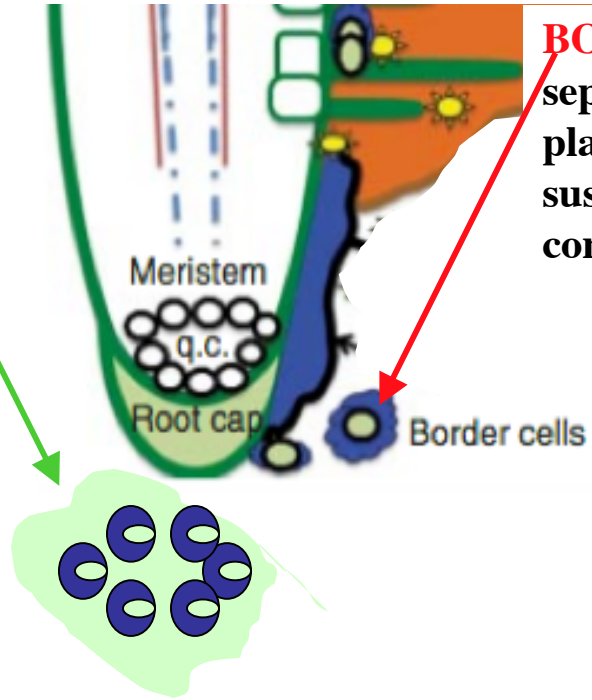
Roles in phytoremediation



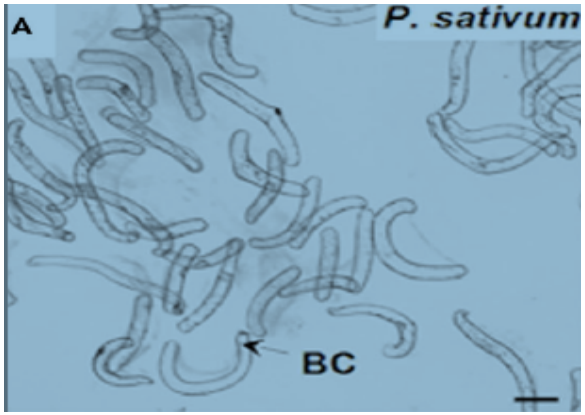
When plan cells behave as bacteria and biofilms:
BORDER CELLS & BORDER LIKE CELLS

BORDER LIKE CELLS: cells separated from roots and organized as sheets of attached cells that remain associated together after their release (Brassicaceae)





BORDER CELLS: cells that separated from the root tips of higher plants and disperse individually into suspension immediately after their contact with water



hundreds/thousands
viable for weeks

From a curiosity to a relevant role in plant-microbe interactions/plant fitness

-. Protection from pathogenic infection: inhibiting growth of fungus and bacteria (mucilage and antimicrobial enzymes -chitinase, peptidase, glucanase- and extracellular DNA)

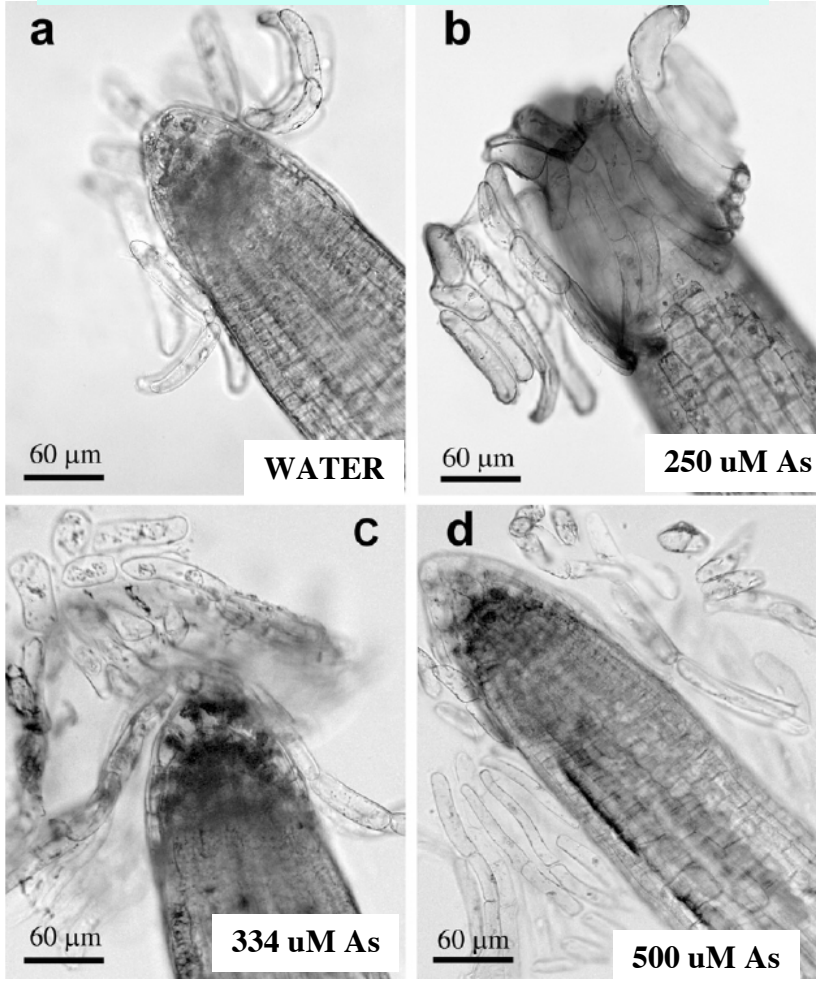
-. Improved root penetration

-. Abiotic stress tolerance (mucilage avoid desiccation, metal immobilization)

Roles in rhizoremediation

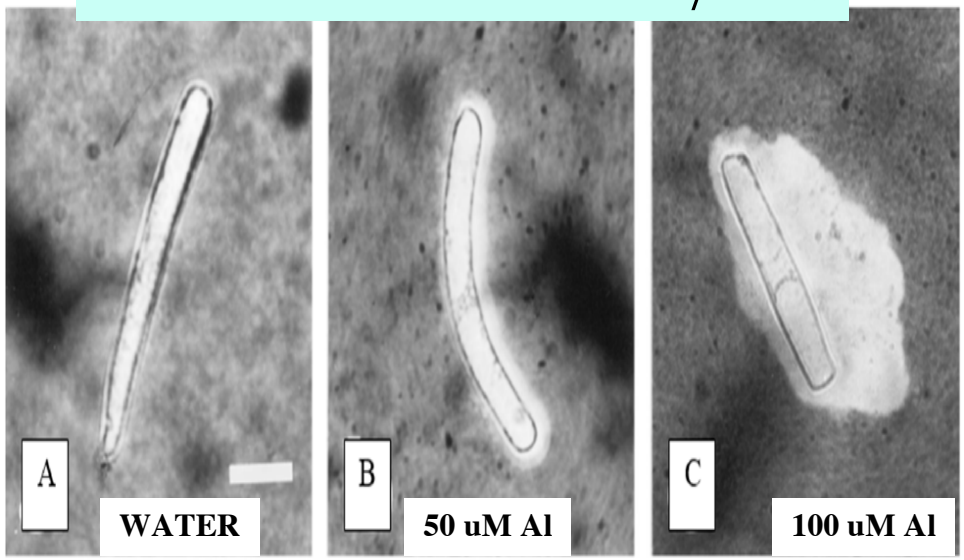
New insights of BC &BLC for Phytoremediation of metals: metal trapping outside of roots

As treated Pteris vittata/ BLC



Border-like cells in P. vittata root apices in control plants (a) and in plants treated for 14 days with 250 uM (b), 334 uM (c), 500 u M (d) of As respectively. Forino et al., 2013

Al treated Pisum sativum/ BLC



Border-like cells of Pisum sativum: Dose dependent induction of extracellular matrix in response to Al for 1h. Hawes et al., 2016

As, Al, Cu, Zn, Ni, Cd, Fe

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pathogens vs PGPBs (Plant Growth Promoting Bacteria)

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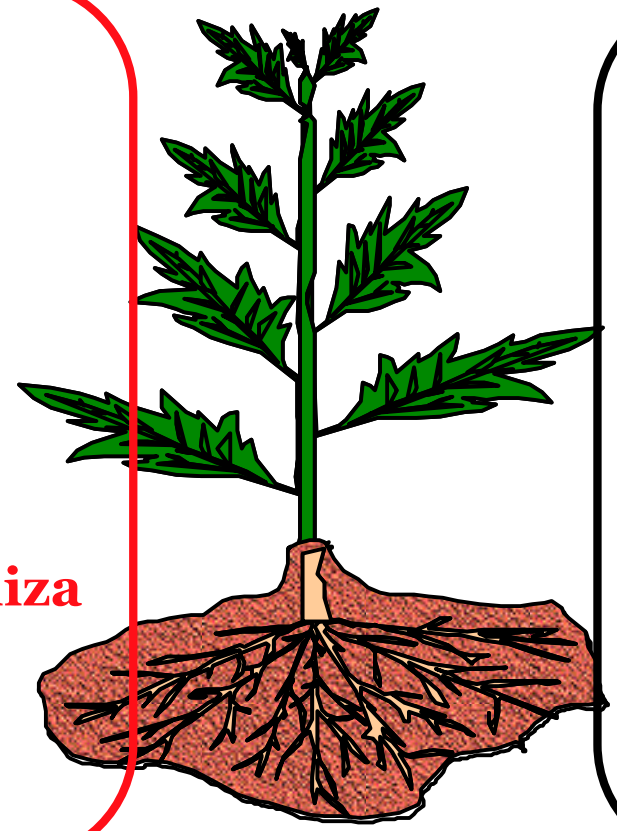
nodule, mycorrhiza



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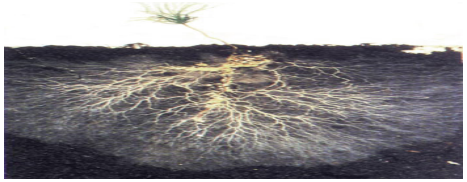

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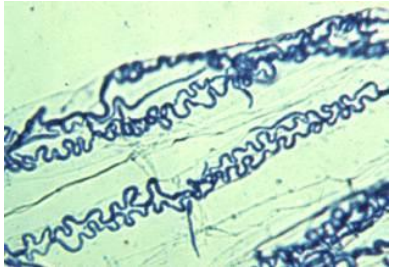
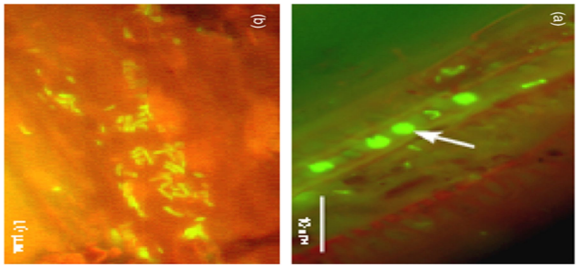


Who? **Endophytes** are non pathogenic microbes colonizing internal tissues of plants:

- -. Internal colonizers & symbionts
- -. Culturable & non culturable

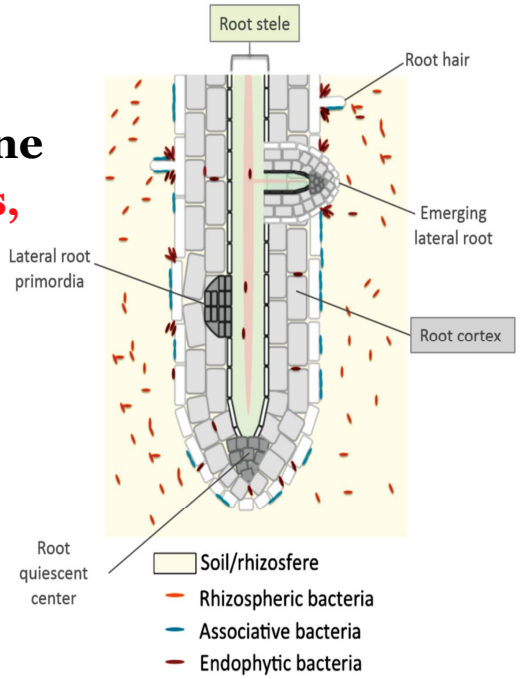
- -. BACTERIA PHYILA: Actinobacteria, Proteobacteria, Firmicutes
- -. FUNGI



How many? endophytes are very common and abundant **10^7 -10^{10} cells/gr of tissue** (10^4 -10^6 cells/gr of soil). Younger plants have more

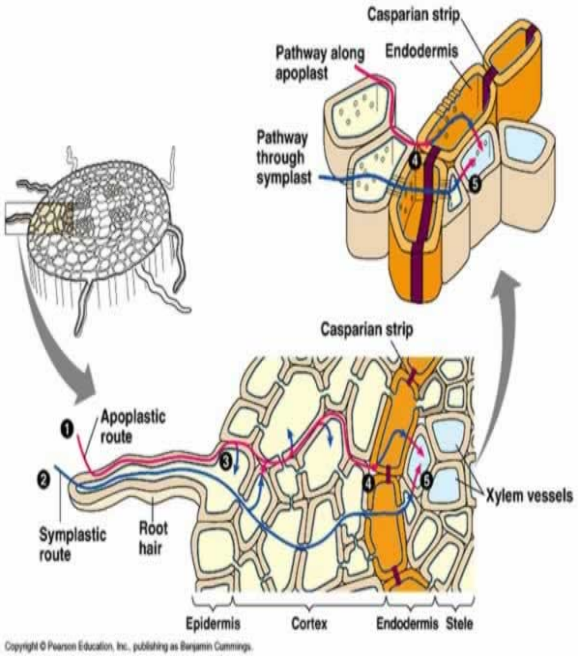
How? Colonization of rhizoplane

Entry: **lateral roots, natural cracks, root tip**



Where?

Root apoplast, cortex, xylem (transpiration stream), leaves, seeds fruits

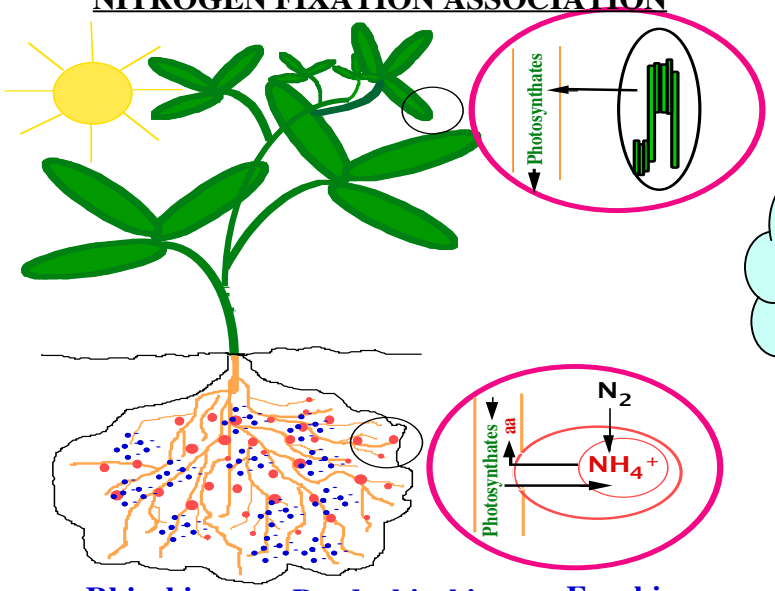


The closest relationship: symbiotic organs

6% of world´s flora

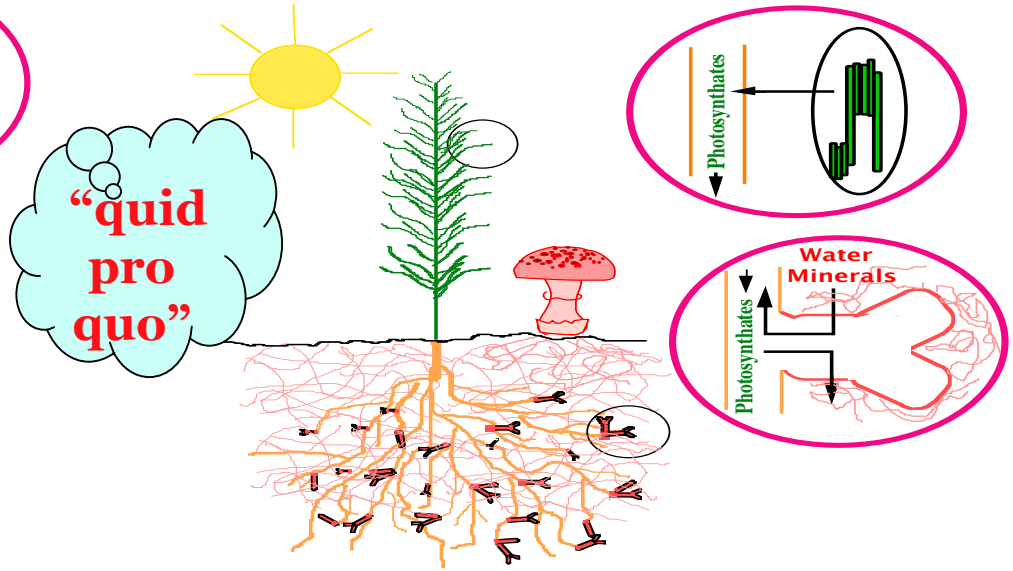
80% of world´s flora

NITROGEN FIXATION ASSOCIATION



Rhizobium Bradyrhizobium Frankia

MYCORRHIZAL ASSOCIATION



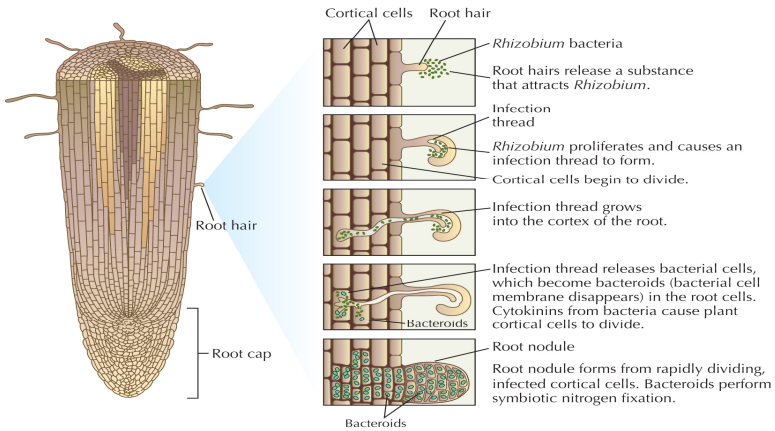
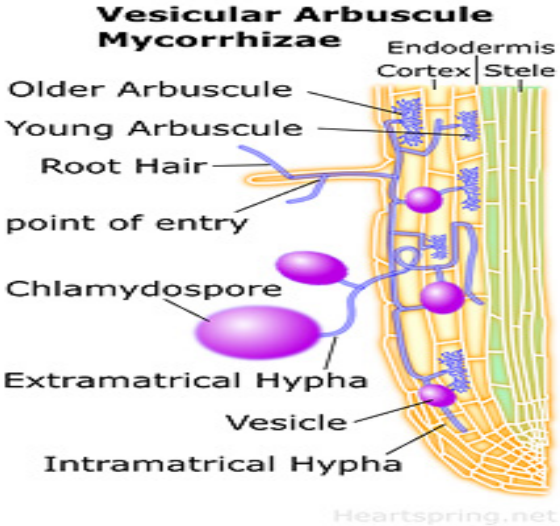
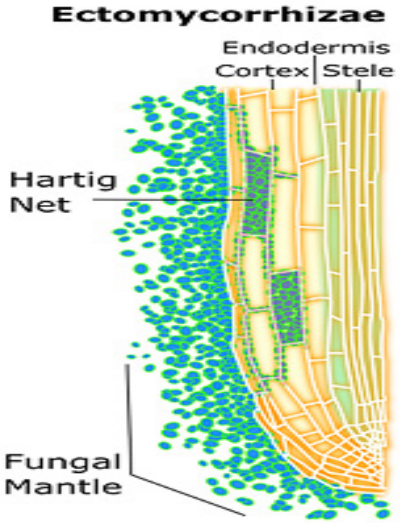


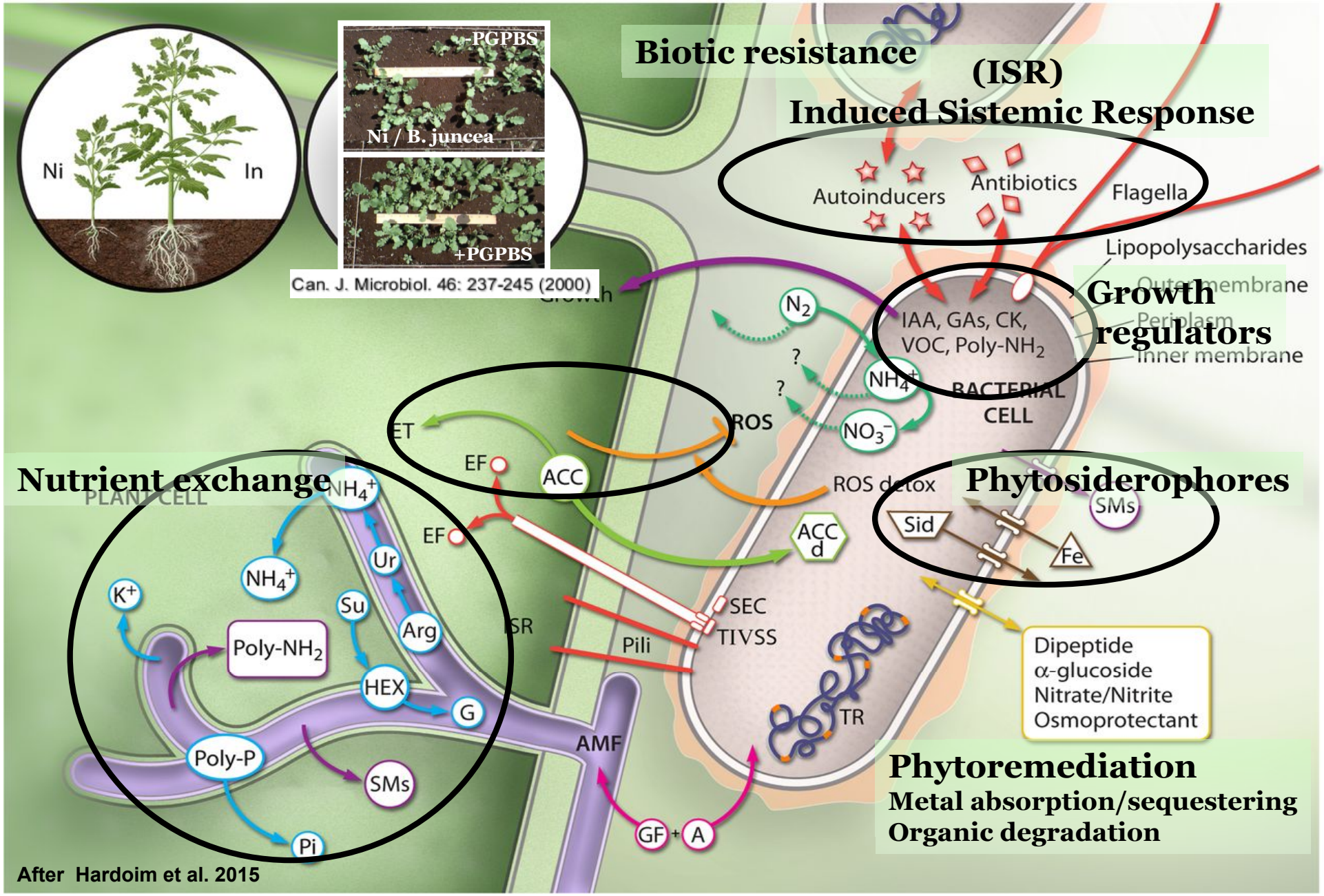
FIGURE 6.26. Symbiosis of *Rhizobium* bacteria with legumes.

Evolution © 2007 Cold Spring Harbor Laboratory Press



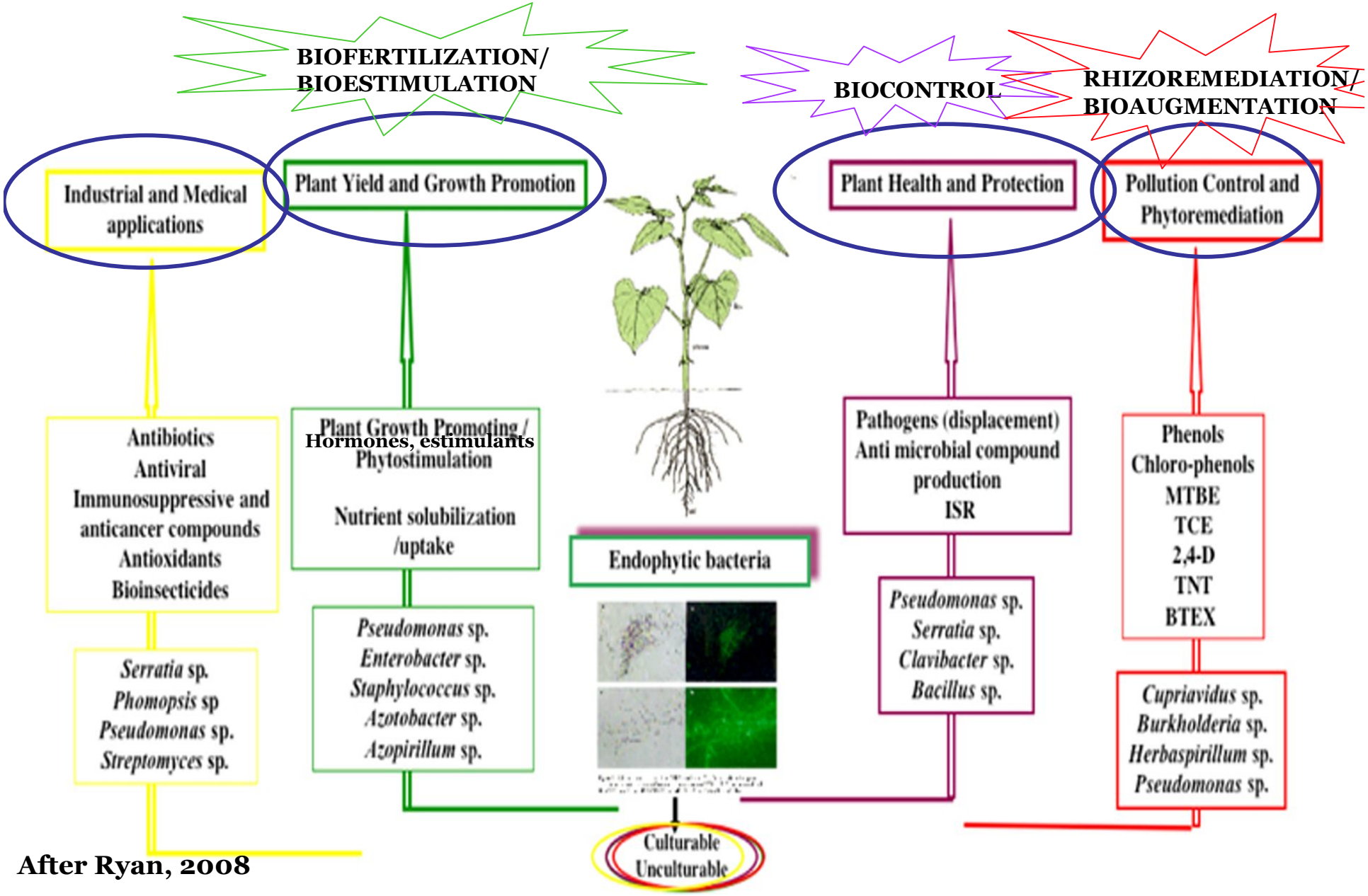


Benefits of quid pro quo relationship



After Hardoim et al. 2015

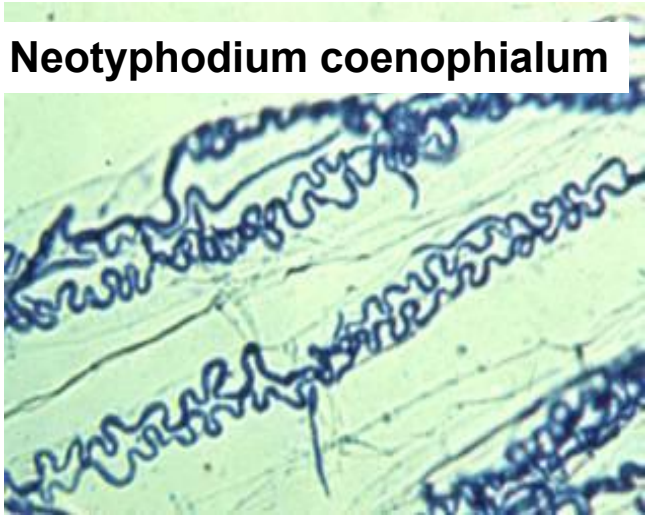
Beneficial endophytic effects & technological applications

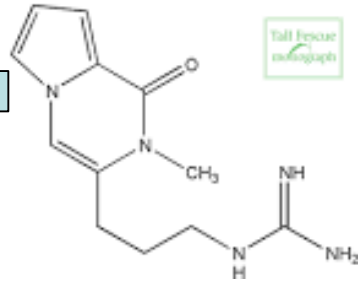


After Ryan, 2008

Developing new strategies for phytoremediation:
Preventing bioaccumulation/biomagnification of contaminants on food web

Symbiosis of the plant Festuca arundinacea and fungal endophyte Neotyphodium coenophialum





Phytoremediator
Metals, organics

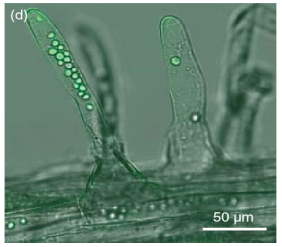
Ioline (alkaloid)

Festuca mycotoxicosis diseases

Feeding deterrent/toxic for herbivorous

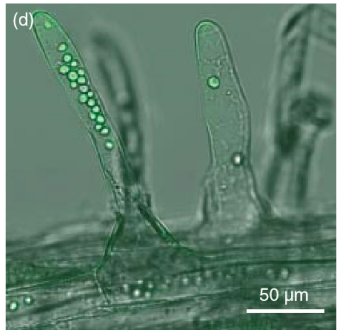
From entomophagy to RHIZOPHAGY

Are plants eating the hand that feed them?

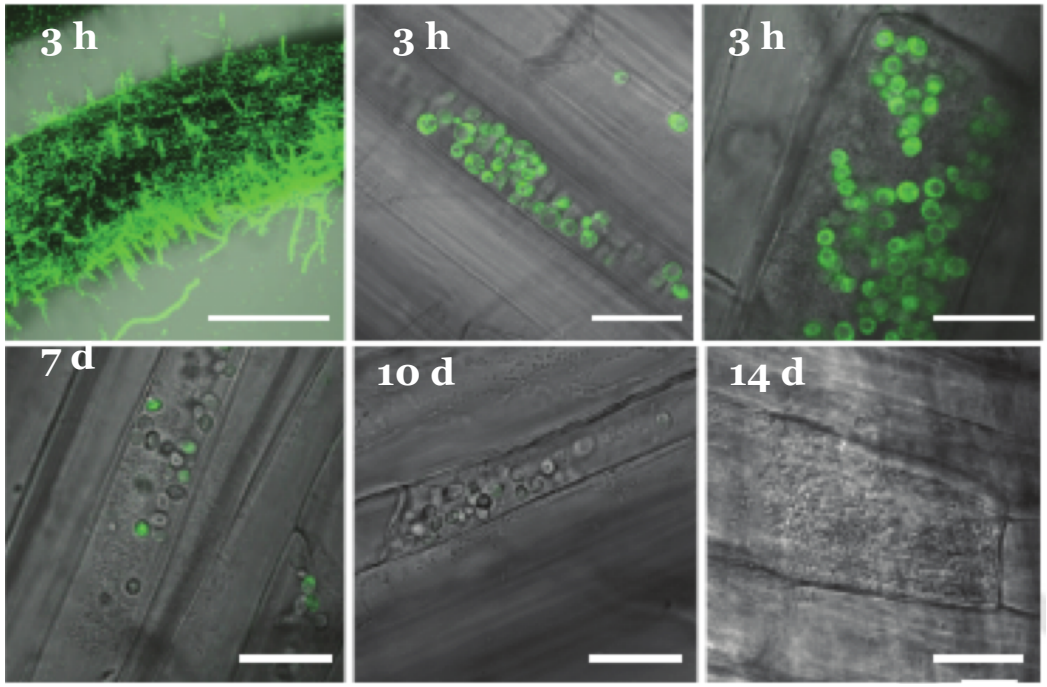


Arabidopsis thaliana
Licopersicon esculentum

E. coli
S. cerevisiae

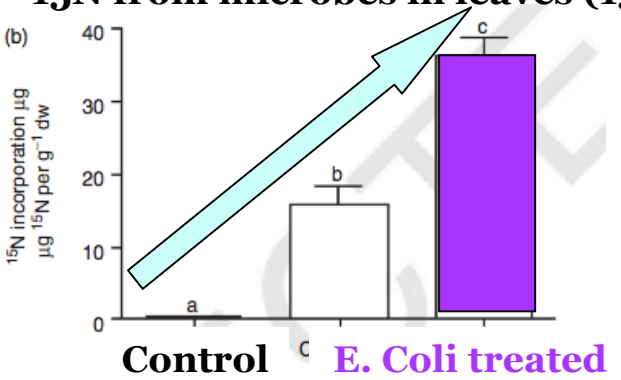


Licopersicon esculentum **S. cerevisiae**



After 14 days tomato plants "digest" yeast of the tissues

15N from microbes in leaves (15 d)



E coli grown with 15N is exposed for 1 h (control 1); 2 h (control t o roots of tomato. After 15 days 15N is detected in leaves of tomato (Treatment)

Paungfoo-lonhienne et al., 2013)

Source of nutrients?
Keep out plant/mutualist bacteria competitors ?
Pathogenic defense?
Microbiome regulation?

The “biased rhizosphere” concept

A prebiotic approach

Microbiota of rhizosphere

BENEFITS

Plant Growth & Health
Disease biocontrol
Xenobiotics detoxification
New industrial products

PROBLEMS

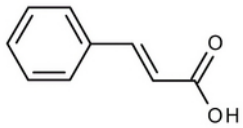
Ability to survive, growth, colonize phytospheres

Persist and compete with indigenous microbiota

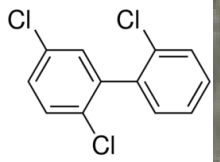
Inefficient performance in the field

Manipulation nutritional compounds secreted to rhizosphere that specifically enhance the growth of desirable microorganisms

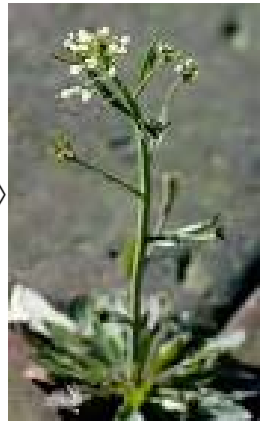
“RHIZOENGINEERING”



Opine/Agrobacterium
Rhizopine/ Rhizobium



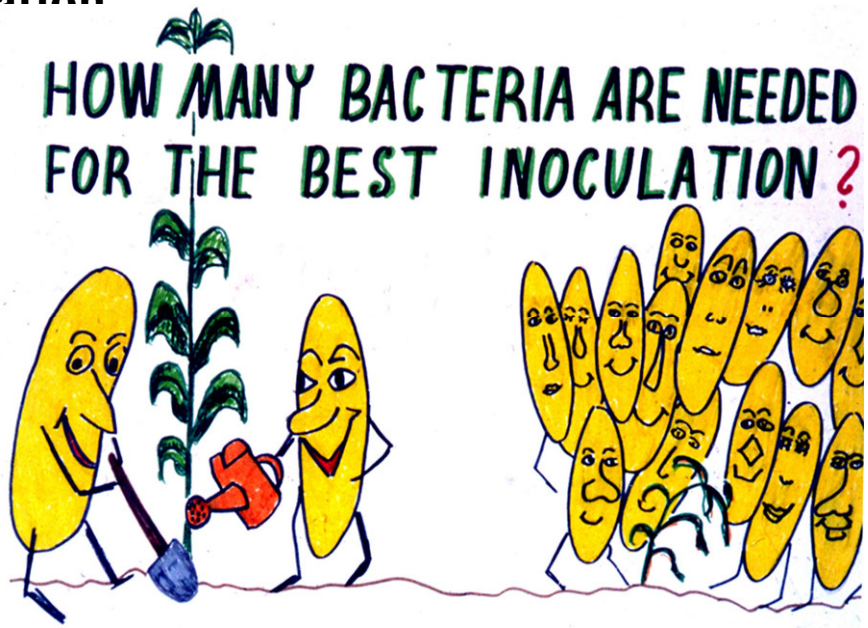
Rizosfera predispuesta en rizoremediación:
Phenylpropanoids/Pseudomonas putida/Arabidopsis/PCBs
Lee et al., 2013



PLANT MICROBIOTA: CHLALLENGES AND OPPORTUNITIES to improve PHYTOREMEDIATION

Improve plant growth and health to improve phytoremediation
Identification microbiome to enhance plant growth, health, tolerance
Microbiome plant comunication
Rhizodeposits
Biofilms and root plant cells in rhizosphere
Endophytes and beyond
Biased rhizosphere
Prebiotics and probiotics in phytoremediation

Influence of Global Change



Message to take home:

"Nobody gives anything for free"

A "quid pro quo" relationship, as that of plants and microbes, seems to be very efficient and convenient for both partners under any environmental conditions even after millions of years.

eskerrik asko, thanks, gracias