Microorganismos e desenvolvimento das plantas

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Curso de Verão – Biotecnologia de Plantas
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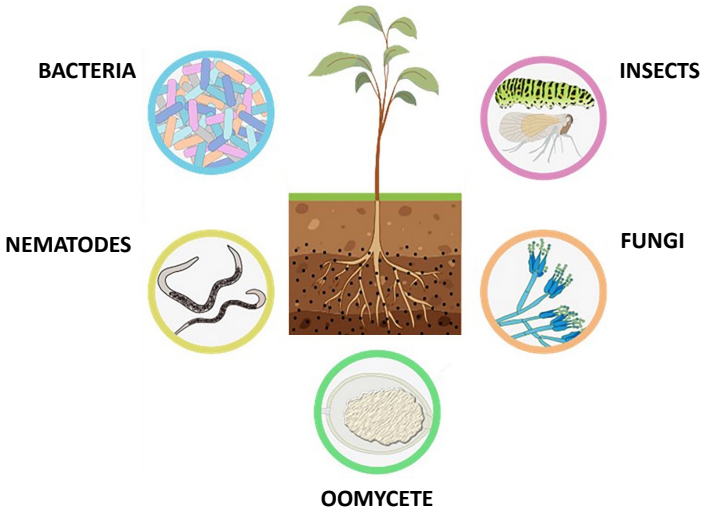
1

Plant-organisms interactions



adapted from Poveda et al. 2021

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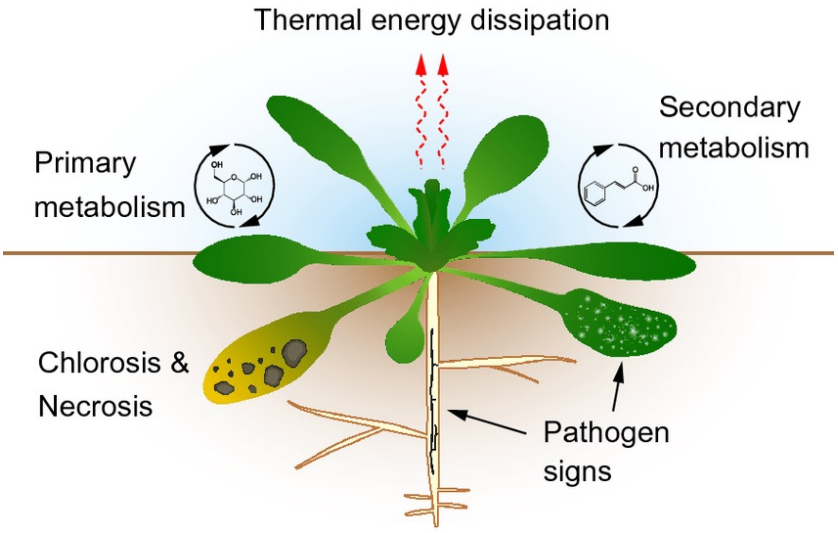
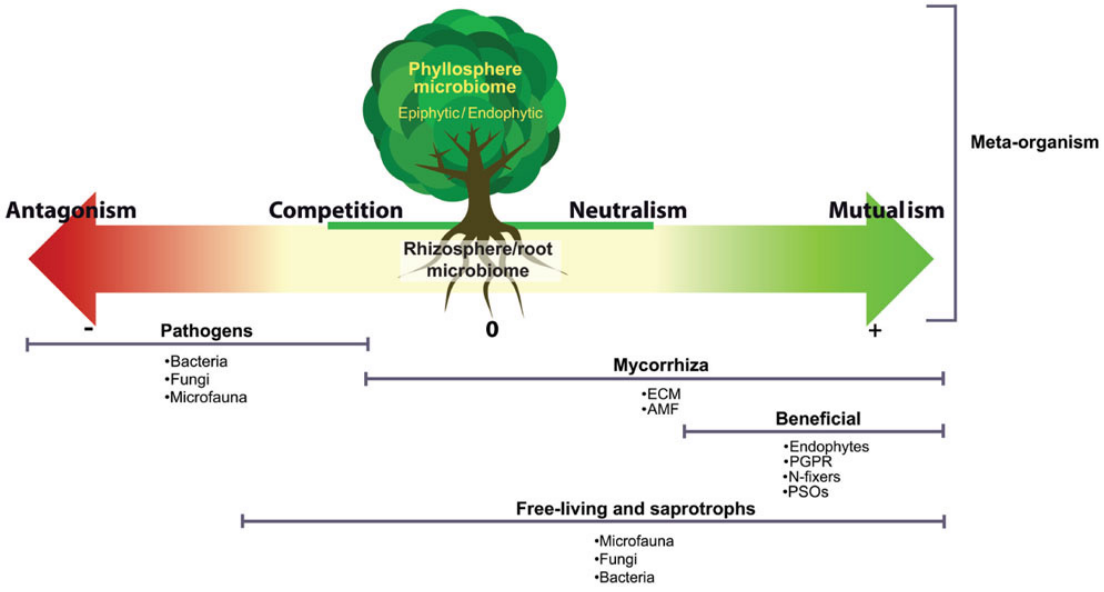
Plant-organisms interactions



adapted from Tanner et al. 2022

5

Plant-organisms interactions



6

Microbiome

The community of microorganisms that can usually be found living together in any given habitat.

Whipps *et al.* 1998

7

Microbiome

Ecological definitions
Definitions based on ecology describe the microbiome following the concepts derived from the ecology of multicellular organisms. The main issue here is that the theories from the macro-ecology do not always fit the rules in the microbial world.
"A convenient ecological framework in which to examine biocontrol systems is that of the microbiome. This may be defined as a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatre of activity "[40].
"...This term refers to the entire habitat, including the microorganisms (bacteria, archaea, lower and higher eurkaryotes, and viruses), their genomes (i.e., genes), and the surrounding environmental conditions. This definition is based on that of "biome," the biotic and abiotic factors of given environments. Others in the field limit the definition of microbiome to the collection of genes and genomes of members of a microbiota. It is argued that this is the definition of metagenome, which combined with the environment constitutes the microbiome. The microbiome is characterized by the application of one or combinations of metagenomics, metabonomics, metatranscriptomics, and metaproteomics combined with clinical or environmental metadata" [25].
"others use the term microbiome to mean all the microbes of a community, and in particular, for the plant microbiome, those microbial communities associated with the plant which can live, thrive, and interact with different tissues such as roots, shoots, leaves, flowers, and seeds" (from Orozco-Mosqueda et al. [41]).
"Ecological community of commensal, symbiotic and pathogenic microorganisms within a body space or other environment" [42].

Organisms/host-dependent definitions
The host-dependent definitions are based on the microbial interactions with the host. The main gaps here concern the question whether the microbial-host interaction data gained from one host can be transferred to another. The understanding of coevolution and selection in the host-dependent definitions is also underrepresented.
"A community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and especially the collection of microorganisms living in or on the human body" [43].
"Human Microbiome Project (HMP): [...] The Human Microbiome is the collection of all the microorganisms living in association with the human body. These communities consist of a variety of microorganisms including eukaryotes, archaea, bacteria and viruses" [44].

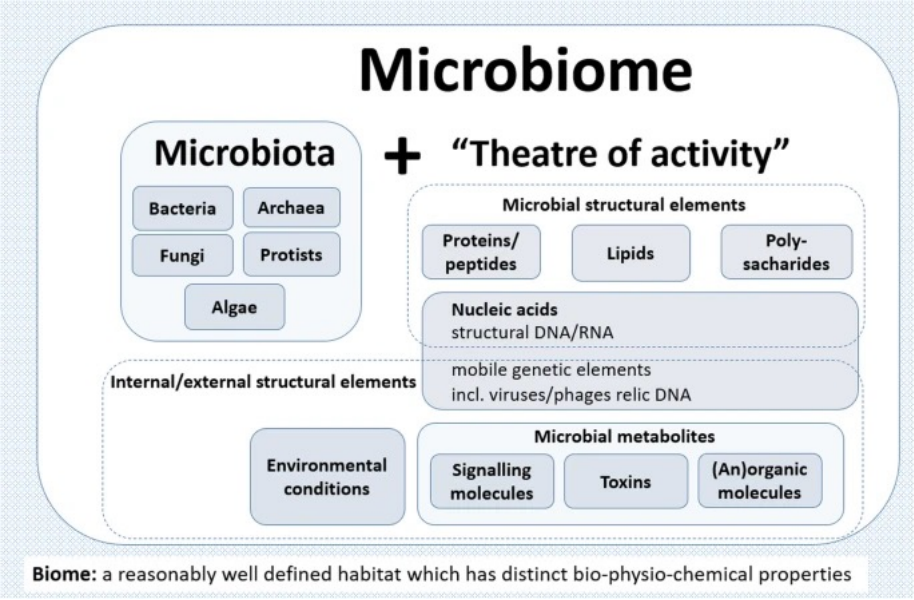
Genomic/ method-driven definitions
There is a variety of microbiome definitions available that are driven by the methods applied. Mostly, these definitions rely on DNA sequence-based analysis and describe microbiome as a collective genome of microorganisms in a specific environment. The main bottleneck here is that every new available technology will result in a need for a new definition.
"The collective genomes of microorganisms inhabiting a particular environment and especially the human body" [43].
"The microbiome comprises all of the genetic material within a microbiota (the entire collection of microorganisms in a specific niche, such as the human gut). This can also be referred to as the metagenome of the microbiota" [45].
"Microbiome is a term that describes the genome of all the microorganisms, symbiotic and pathogenic, living in and on all vertebrates. The gut microbiome is comprised of the collective genome of microbes inhabiting the gut including bacteria, archaea, viruses, and fungi" [46].
"Different approaches to define the population provide different information. a | Microbiota: 16S rRNA surveys are used to taxonomically identify the microorganisms in the environment. b | Metagenome: the genes and genomes of the microbiota, including plasmids, highlighting the genetic potential of the population. c | Microbiome: the genes and genomes of the microbiota, as well as the products of the microbiota and the host environment" [47].
"Totality of genomes of a microbiota. Often used to describe the entity of microbial traits (=functions) encoded by a microbiota." [48]

Combined definitions
There are some microbiome definitions available that fit several categories with their advantages and disadvantages.
"A microbiome is the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space" [49].
"The microbiome is the sum of the microbes and their genomic elements in a particular environment" [50].
"The genes and genomes of the microbiota, as well as the products of the microbiota and the host environment" [51].

Berg et al. 2020

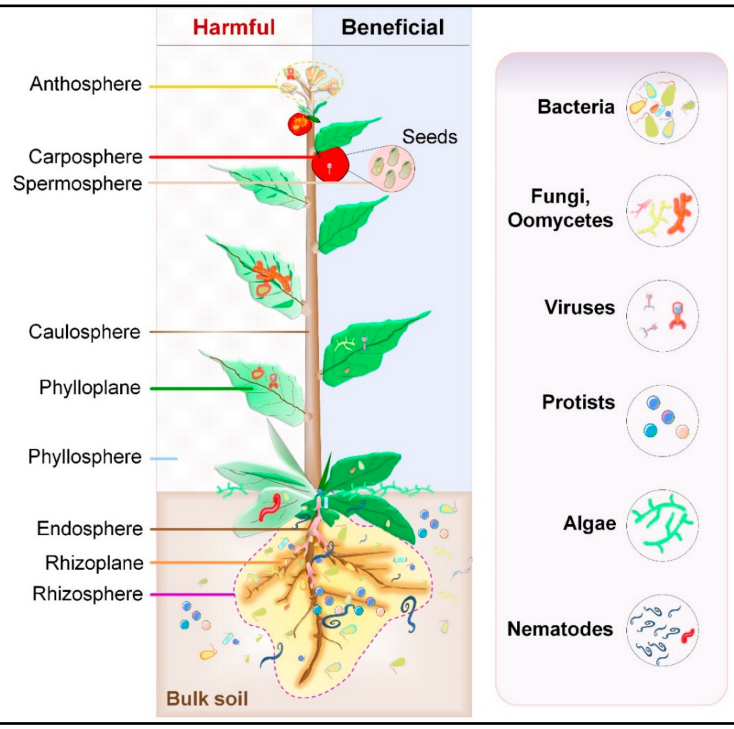
8

Microbiota/Microbiome



adapted from Berg et al. 2020

9



adapted from Shelake et al. 2019

10

Microbiome research

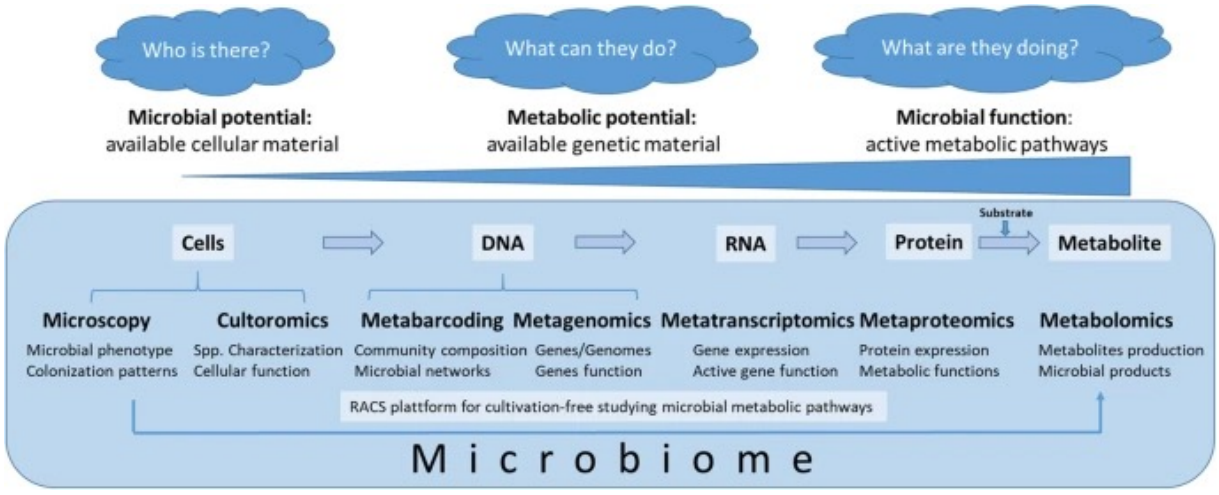
Method Innovations	Important Discoveries	
		17th century
1670 Microscopy	1670 discovery of microorganisms (Anthony van Leuwenhook "Father of Microbiology")	
	1729 classification of plants and fungi (Pier Antonio Micheli)	
	1796 first vaccination (Edward Jenner)	
	1837 yeast in alcoholic fermentation (Charles C. de la Tour, Friedrich T. Kützung and Theodor Schwann)	
1857 cultivation based approaches	1857-1855 Pasterisation, fermentation, vaccine against rabies (Louis Pasteur)	
	1875 foundation for bacteriological taxonomy (Ferdinand Cohn)	
	1884 Robert Kochs´ postulates	
1911 fluorescence microscopy	1888-begin of microbial ecology by Sergei Winogradsky (nitrification, nitrogen-fixation, soil microbiology, cycle-of-life)	
1911 mass spectrometry	1892 tobacco-mosaik-virus extraction from leafs (Dmitri I. Ivanovski and Martinus Beijerinck)	
	1922 chemolithotrophy (Sergei Winogradsky)	
	1904 the rhizosphere concept (Lorenz Hiltner)	
	1928 transformation of the genetic information to their offsprings (Frederick Griffith)	
1931-38 electron & scanning-transmission microscopy	1928 discovery of antibiotics (Alexander Fleming)	
	1944 DNA as carrier of genetic information (Oswald Avery, Colin MacLeod, Maclyn McCarty)	
	1946 'sexual reproduction' of bacteria (Joshua Lederberg and Edward Tatum)	
	1953 3D-double-helix structure (James Watson and Francis Crick)	
1969 *in situ* Hybridization		
1970s HPLC	1970 central dogma of molecular biology (Francis Crick)	
1975 DNA array/colony hybridization		
1977 Sanger sequencing and molecular fingerprinting	1977 discovery of Archaea (Carl Woese and George E. Fox) and first full genome sequence of a virus	
	1982 discovery of prions (Stanley B. Prusiner)	
1983 PCR technique		
1988 fluorescence-*in situ*-hybridization	1991 theory of the holobiont (Eugene Rosenberg and Ilana Zilber-Rosenberg)	
	1993 discovery of the complex structures of biofilms (Hans-Curt Flemming)	
1993 quantitative real-time PCR	1995 first Genome of *Haemophilus influenzae* (John C. Venter and colleagues)	
1995 full-cyle rRNA approach		
2005 next-generation sequencing	2005 HMP: Human Microbiome Project	
2008/9 third-generation sequencing	2008 TerraGenome: Reference Soil Metagenome Project	
	2010 EarthMicrobiome Project	
		21th century

Antoni van Leeuwenhoek

adapted from Berg et al. 2020

11

Microbiome research



adapted from Berg et al. 2020

12

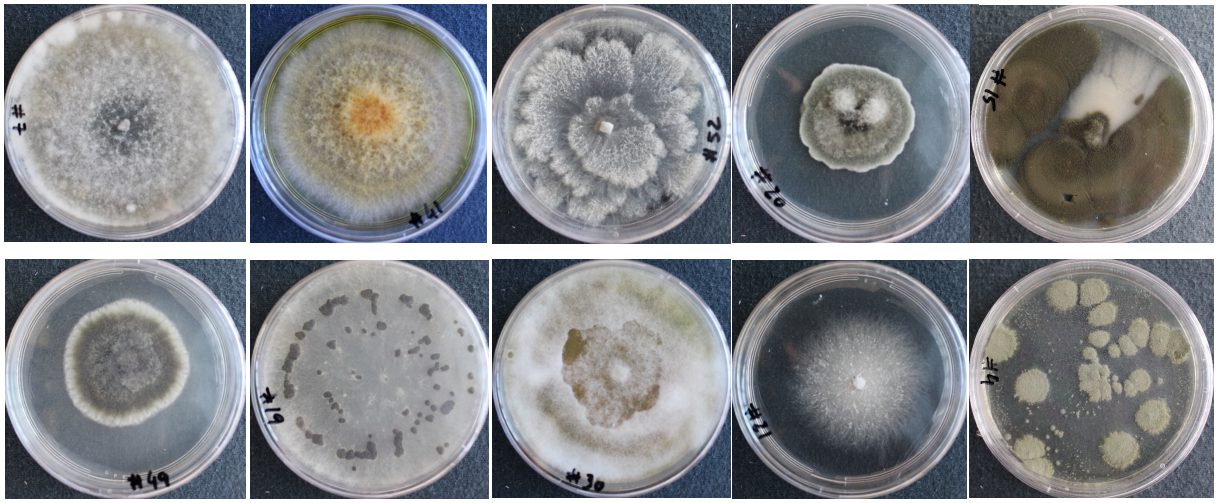
Who is there?

Cultura e isolamento

13

Who is there?

Identificação morfológica



14

Who is there?

Roots	Twigs	Leaves
Aureobasidium pullulans	*Aureobasidium pullulans*	*Alternaria* sp.
Phaelocephala fortini	*Botrytis cinerea*	*Aureobasidium pullulans*
Penicillium sp.	*Diaporthe viticola*	*Cladosporium* sp.
Thricoderma sp.	*Dicostroma fuscellum*	*Diaporthe* sp.
Thricoderma atroviride	*Epicoccum nigrum*	*Pyrenopeziza* sp.
Thricoderma asperellum	*Glomerela cingulata*	*Hypoderma rubi*
Umbelopsis sp.	*Pyronema* sp.	*Lophodermium conigenum*
	Sordarya sp.	*Mycosphaerella aurantis*
		Mycosphaerella punctiformis
		Mycosphaerella sp.
		Phoma sp.
		Saccothecium sp.

15

Molecular tools

Ribotyping

Amplified ribosomal DNA restriction analysis (ARDRA)

Random amplified polymorphic DNA (RAPD)

Amplified fragment length polymorphism (AFLP)

Pulse field gel electrophoresis (PFGE)

Repetitive extragenic palindromic PCR (Rep-PCR)

Denaturing (D)/temperature (T) gradient gel electrophoresis (DGGE/TGGE)

Terminal (T)-restriction fragment length polymorphism (T-RFLP)

Microarrays

Real-time PCR

Multilocus sequence typing (MLST)

Whole-genome sequencing (WGS)

16

Molecular tools

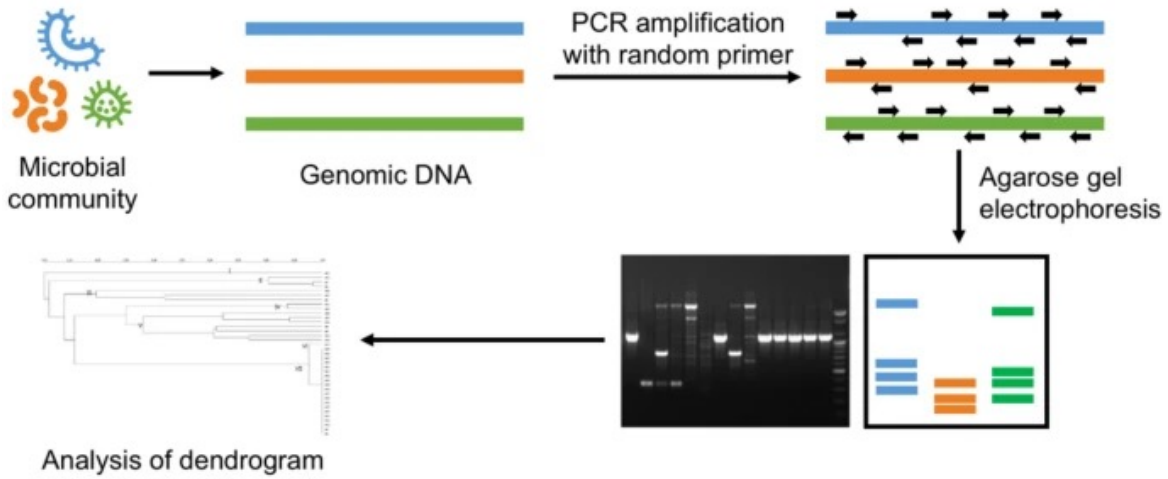
Feature	*Plasmid analysis*	*(IS) RFLPs*	*Ribotyping*	*PFGE*	*PCR-RFLP*	*RAPD-PCR*
Typeability	Many	All	All	All	All	All
Repeability[4]	Moderate	Excellent	Excellent	Excellent	Excellent	Moderate
Reproducibility[4]	Moderate	Good	Excellent	Excellent	Excellent	Moderate
Discriminatory power[4]	Poor	Moderate to excellent[2]	Moderate to excellent[2]	Excellent	Poor to moderate[2]	Good
Stability[4]	Moderate	Good	Good	Good	Good	Moderate
Ease of interpretation of data generated[4]	Moderate	Moderate	Moderate to good	Moderate	Good	Moderate
Ease of use[4]	Moderate	Poor	Poor to moderate[4]	Poor	Good	Good
High throughput	No	No	No	No	No	Yes
Cost[4]	Low	Moderate	High	Moderate	Low to moderate	Low
Time required (days)[3]	1	3-5	1 to 3-5	3	1-2	1

[1]If automated. [2]The discriminatory power may perform differently based upon clonality of organisms (for example, some serotypes of *Salmonella* or some clones of MRSA). [3]The approximate number of days to get typing results is estimated by excluding the interval of time to

adapted from Ranjbar et al. 2014

17

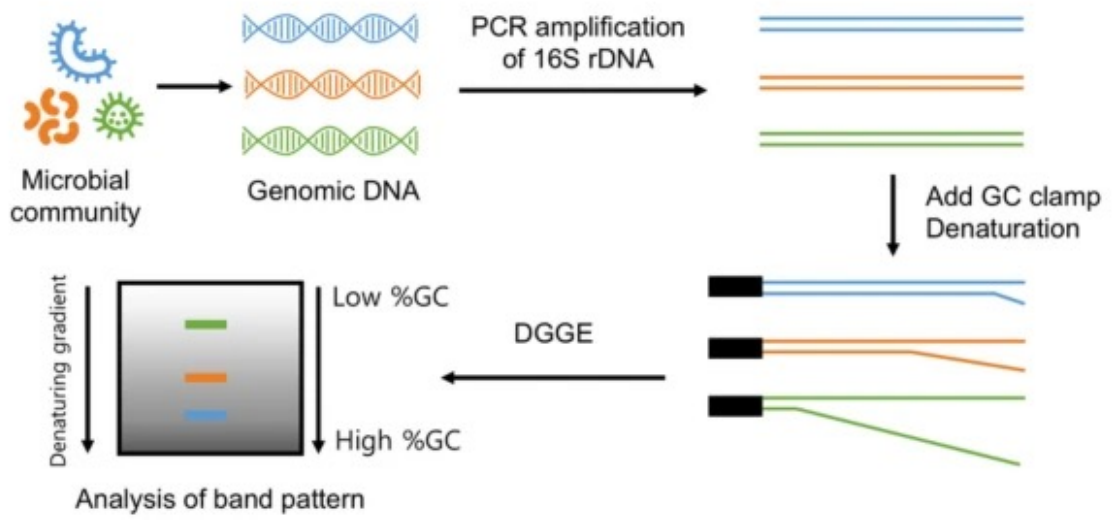
Random amplified polymorphic DNA (RAPD)



adapted from Sharma et al. 2020

18

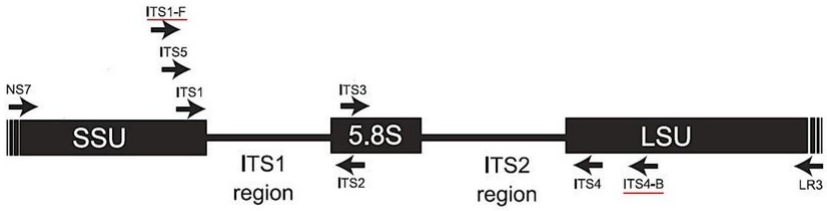
Denaturing gradient gel electrophoresis (DGGE)

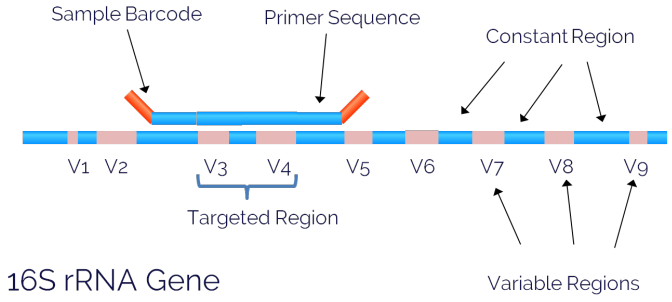


adapted from Sharma et al. 2020

19

16S / 18S / ITS amplicon sequencing





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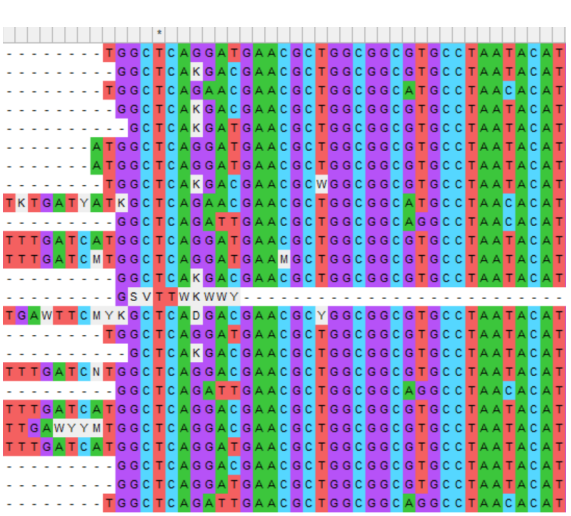
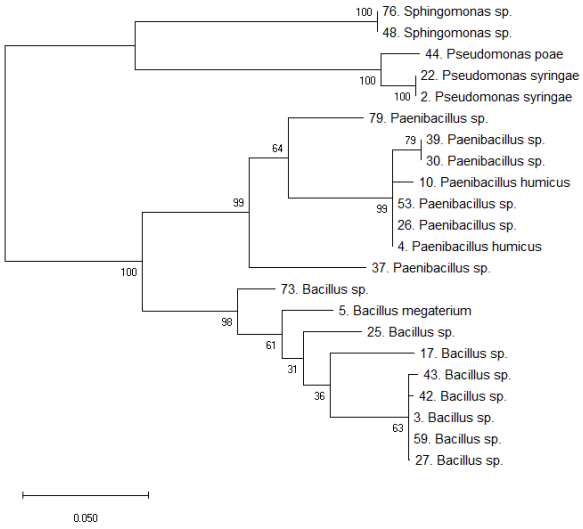
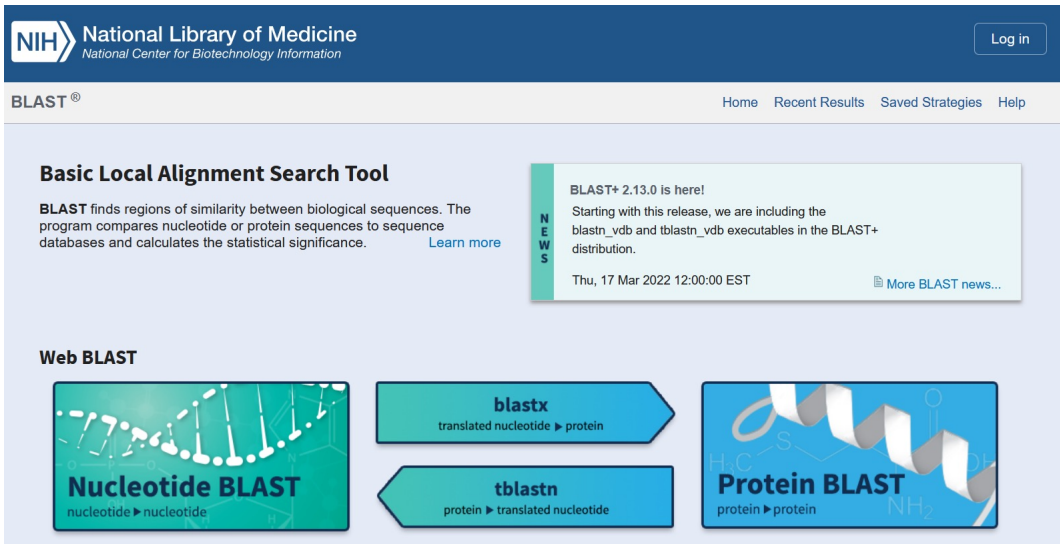
16S / 18S / ITS amplicon sequencing





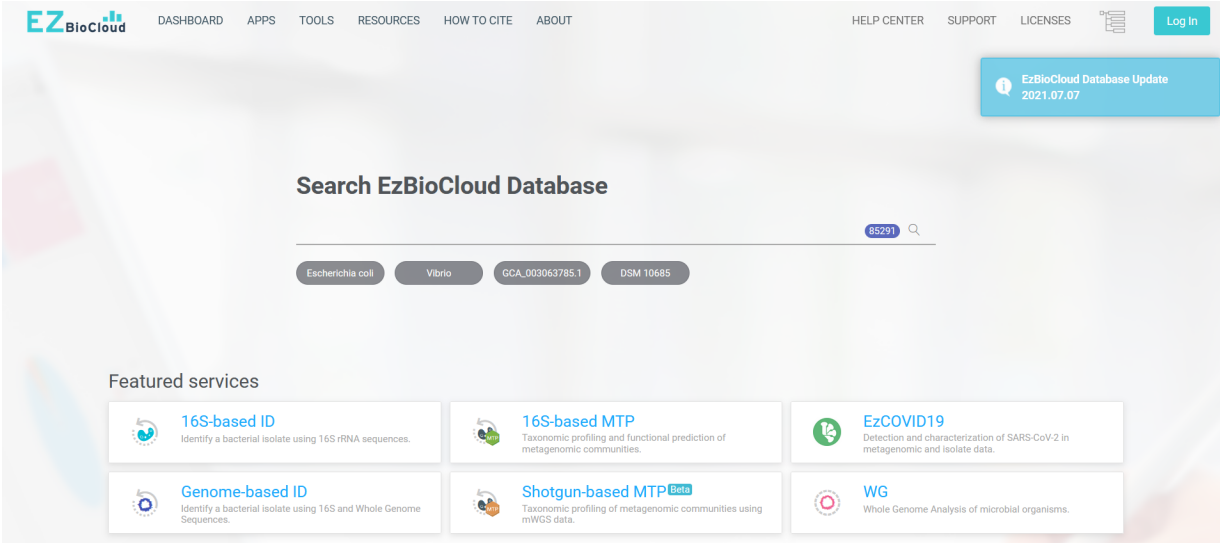
21

Databases



22

Databases



23

Databases

JGI MycoCosm
THE FUNGAL GENOMICS RESOURCE

FungiDB Release 58 23 Jun 2022
Fungal & Oomycete Informatics Resources

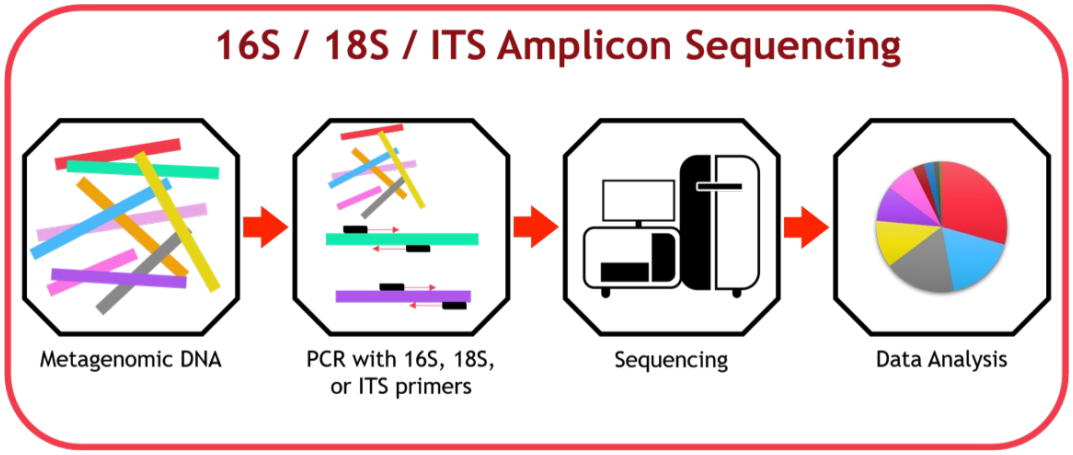
MiST
Microbial Signal Transduction Database 3.0

FUSARIOID-ID database - Food, Fibre & Health
WESTERDIJK FUNGAL BIODIVERSITY INSTITUTE
A database for fusarioid fungi

adapted from Hirt et al. 2020

24

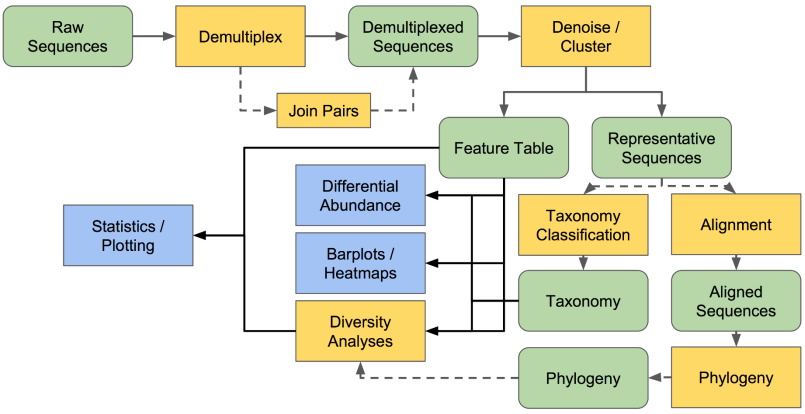
16S / 18S / ITS amplicon sequencing



https://www.biobasic-asia.com/16s-18s-its-amplicon-sequencing/

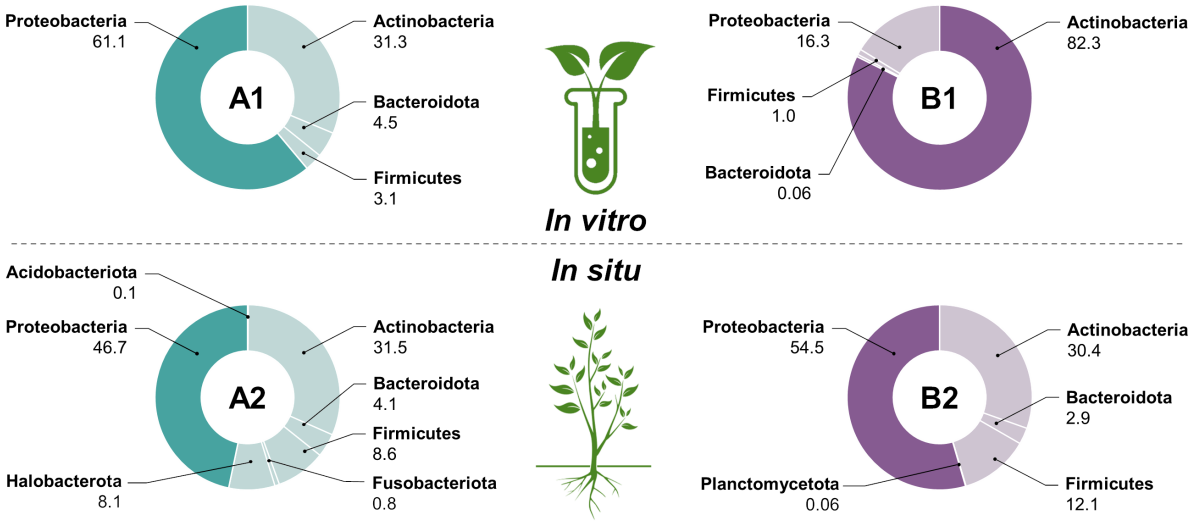
25

16S / 18S / ITS amplicon sequencing



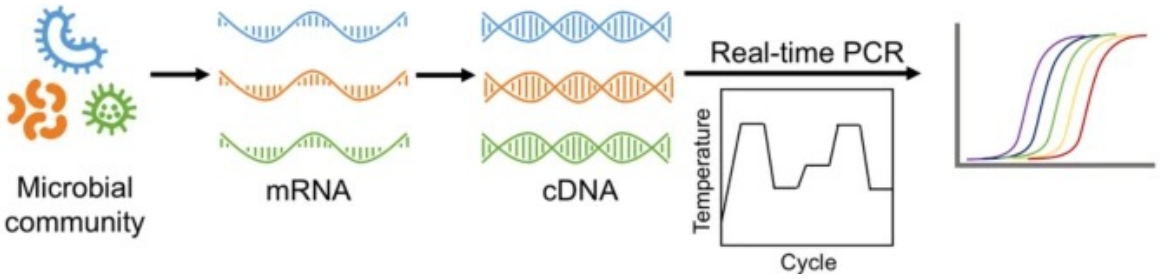
26

16S sequencing



27

Real-time PCR



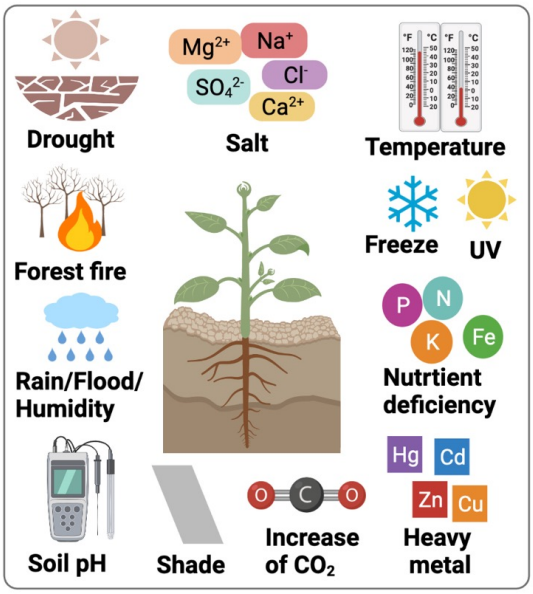
It is used to analyze the gene expression and comparative amount of DNA.

Absolute quantification (standard curve) or relative quantification (comparative threshold method).

adapted from Sharma et al. 2020

28

Factors affecting plant microbiota



https://mobile.twitter.com/kashtanlab

29

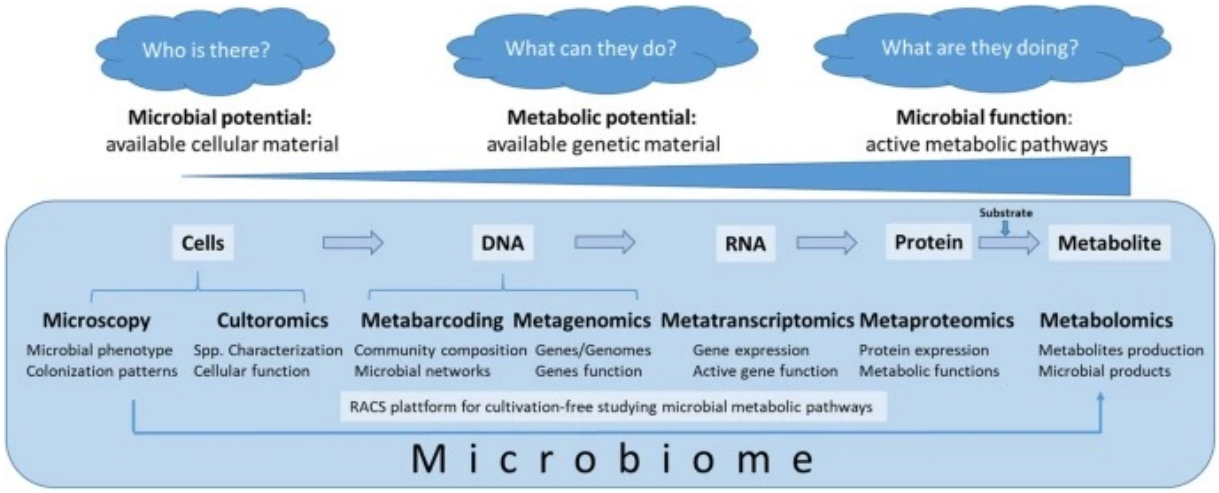
Factors affecting plant microbiota

Host environment	Microbiota
Soil	
• Variable pH 3.5–10 • Oxygenated • Low nutrients	• High diversity (4×10^3–5×10^4 different bacterial species) • High numbers (10^6–10^{10} bacteria/g soil) • High competition with other microbiota and organisms (arthropods, nematodes, earth worms, …)
Rhizosphere	
• Neutral pH • Oxygenated • Mucilage and root exudates (sugars, amino acids, organic acids, …)	• Medium diversity • High numbers (10^6–10^{10} bacteria/g rhizosphere soil) • Low competition with other soil microbiota and organisms
Endosphere	
• Acidic pH • Low O_2 • High nutrient access	• Low diversity • Low numbers (10^4–10^8 bacteria/g plant) • No competition with soil microbiota and other soil organisms

adapted from Hirt et al. 2020

30

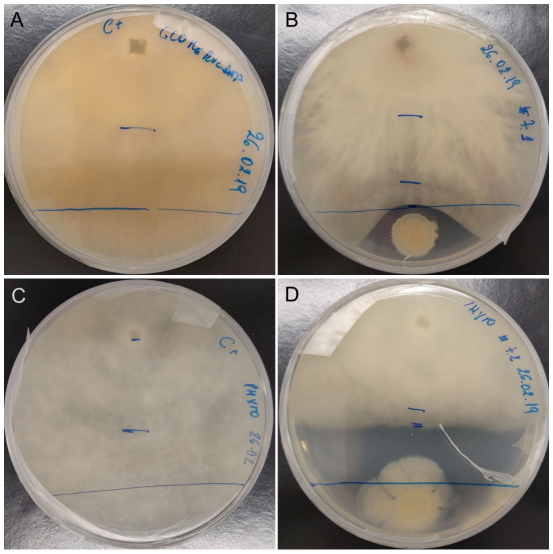
What they can do?

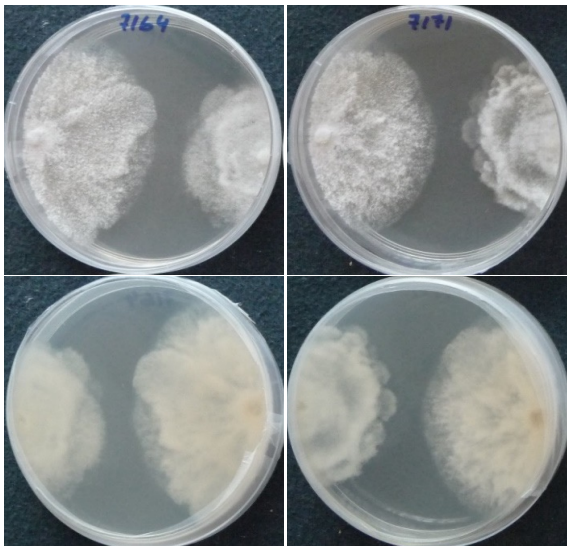


adapted from Berg et al. 2020

31

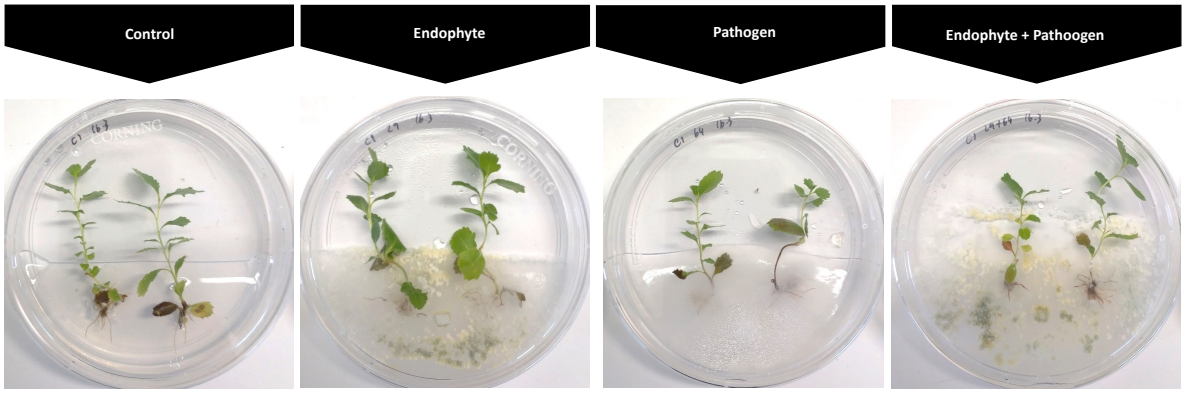
Antagonism





32

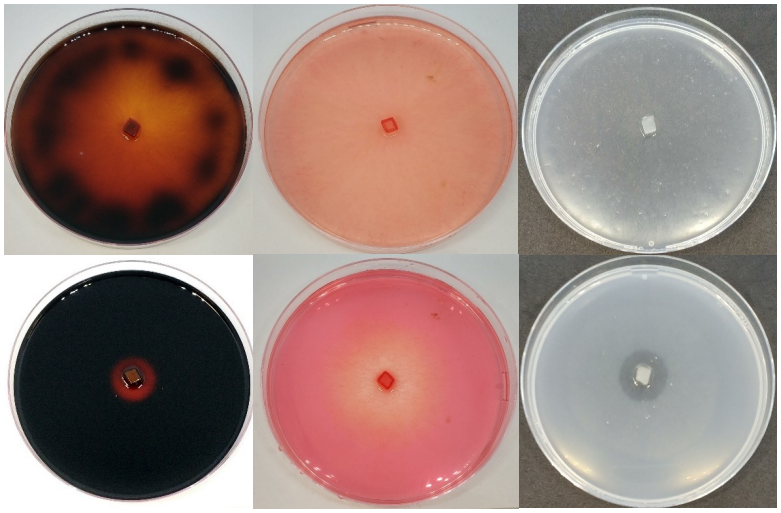
Antagonism (Plant + Endophyte + Pathogen)

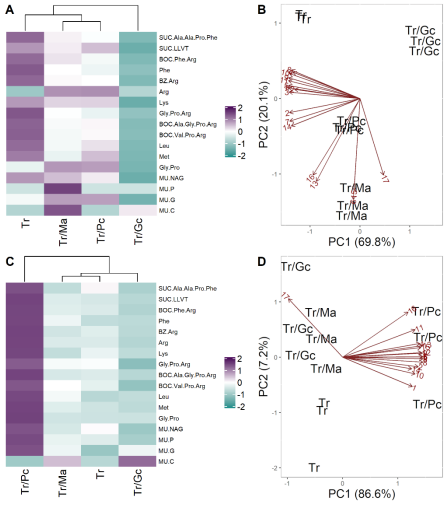


33

Enzimes

Amylase Cellulase Protease



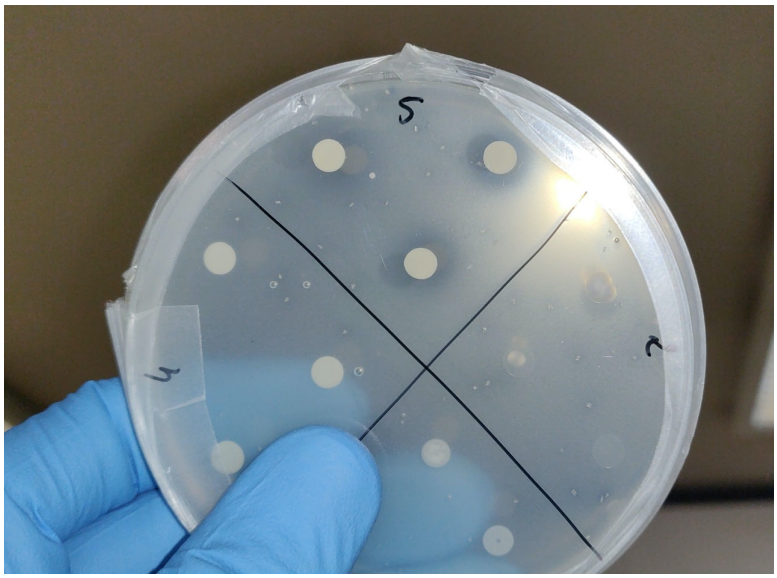


34

Ammonia production

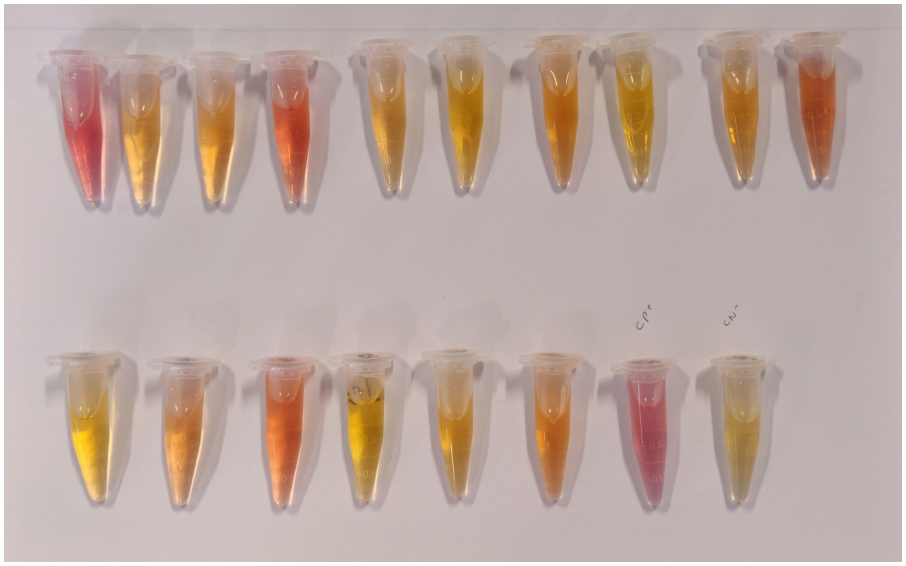
35

Phosphate solubilization



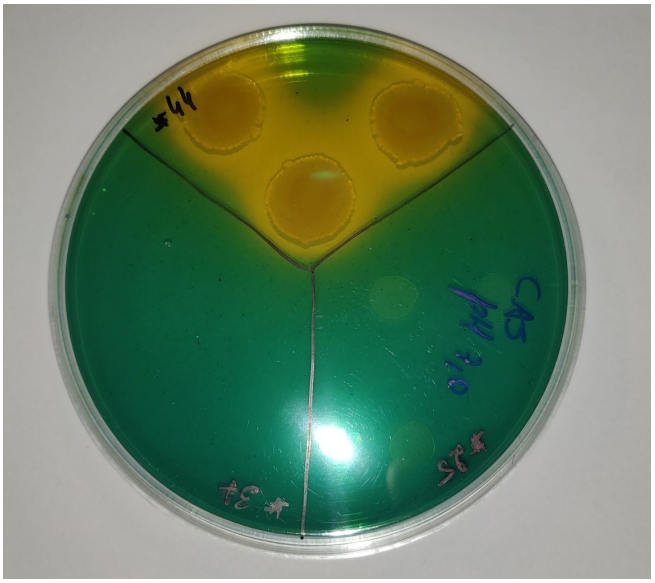
36

IAA production



37

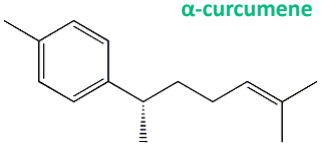
Siderophore production



38

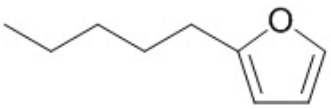
Volatile compounds

α-curcumene



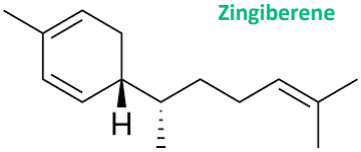
Common on *Curcuma* sp. (Zingiberaceae)

2-pentylfuran



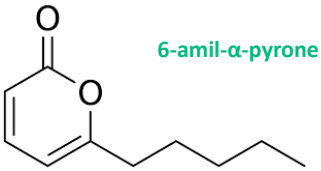
Antifungal properties, produced by several fungi species

Zingiberene



30% of the essential oils in ginger rhizomes.
Gives ginger its distinct flavoring.

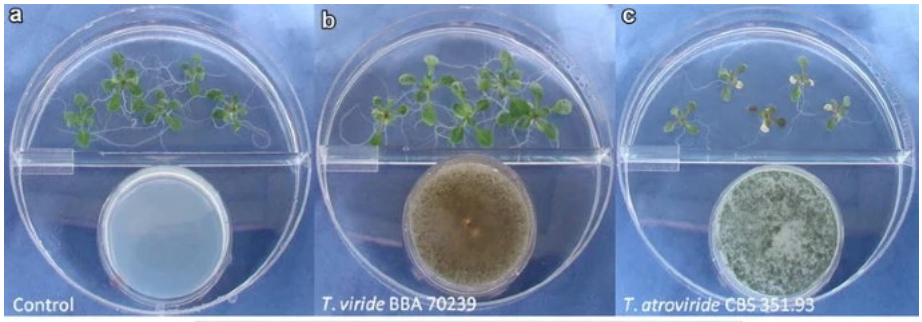
6-amil-α-pyrone

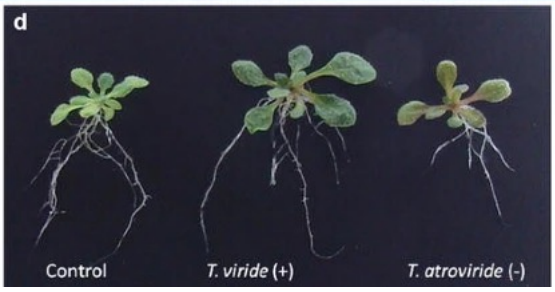


Characteristic coconut aroma.
Produced biologically by *Trichoderma* species.
Important for plant-fungi communication (elicitor)

39

Volatile compounds

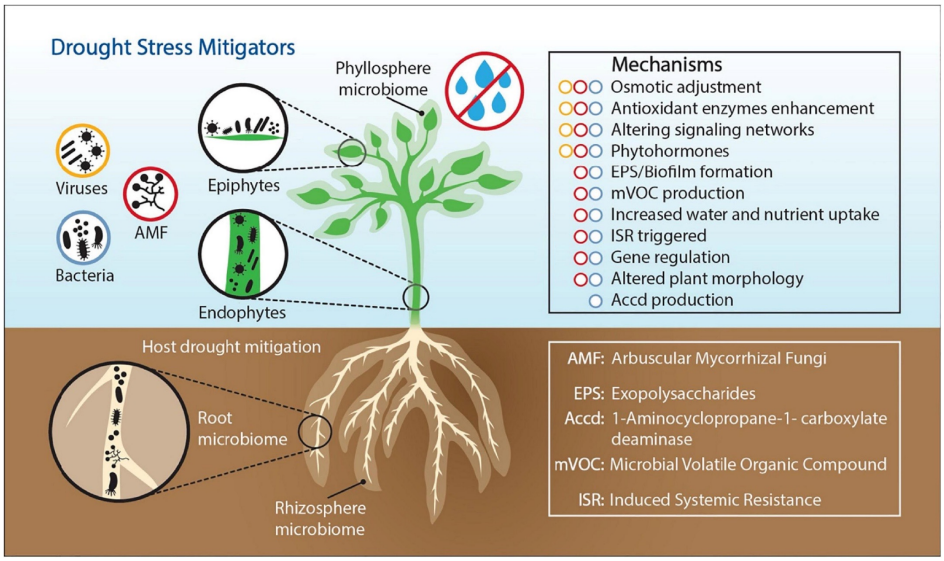




Lee et al. 2016

40

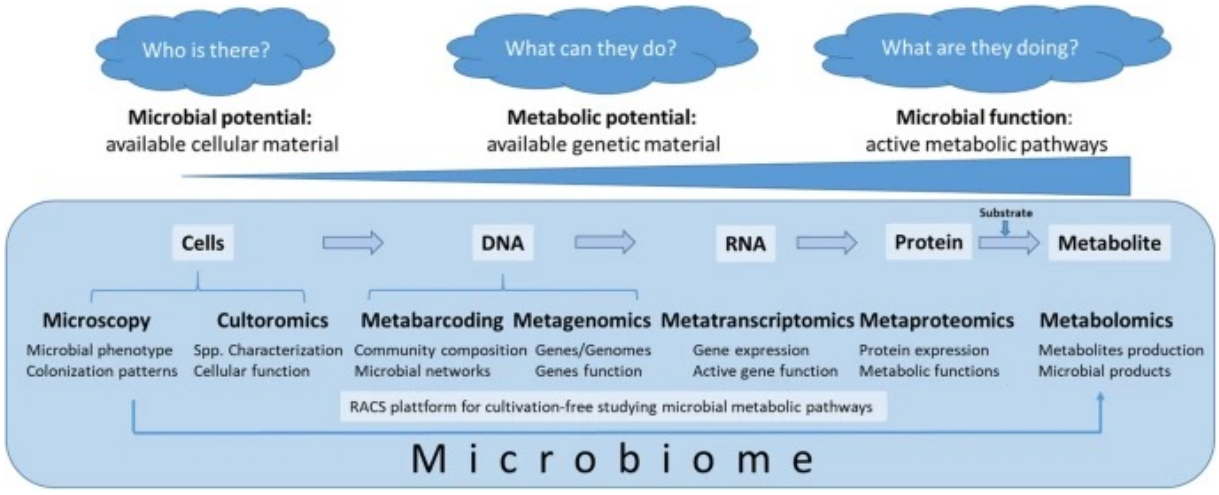
What they can do?



adapted from Poudel et al. 2021

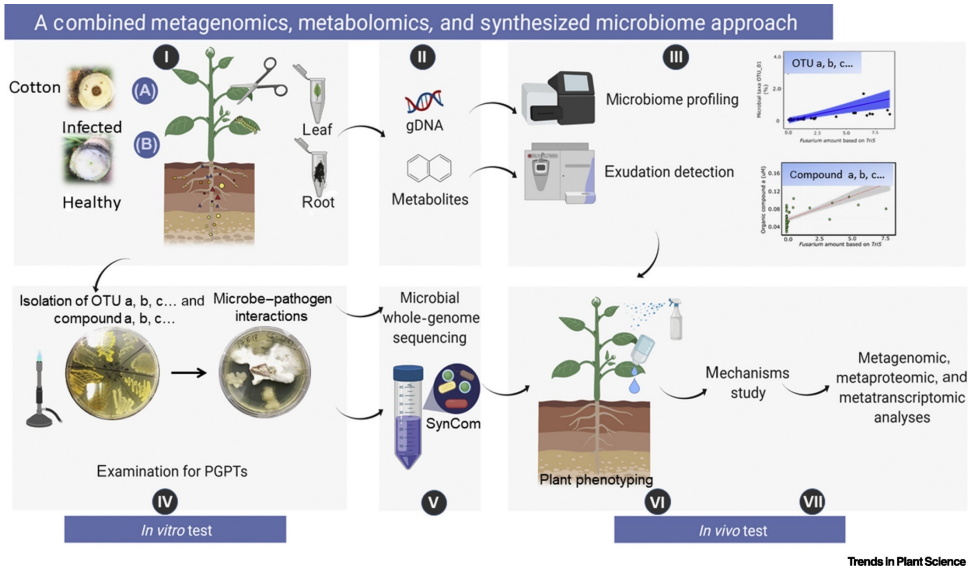
41

What are they doing?



42

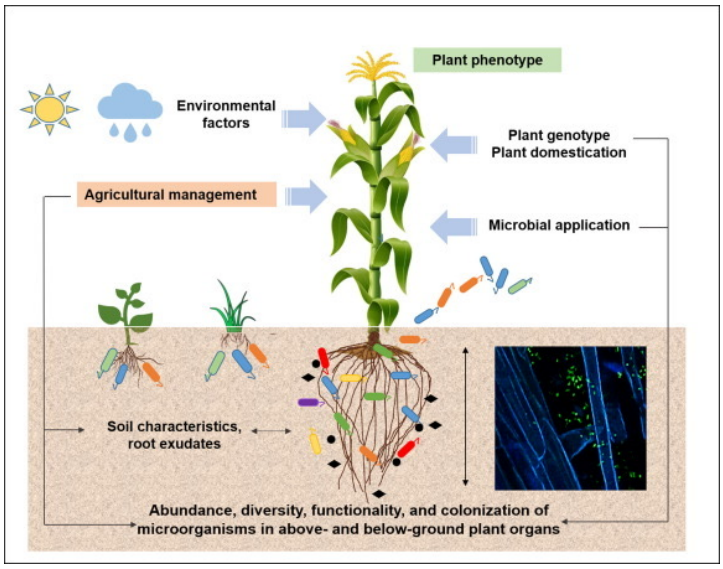
What are they doing?



Liu et al. 2020

43

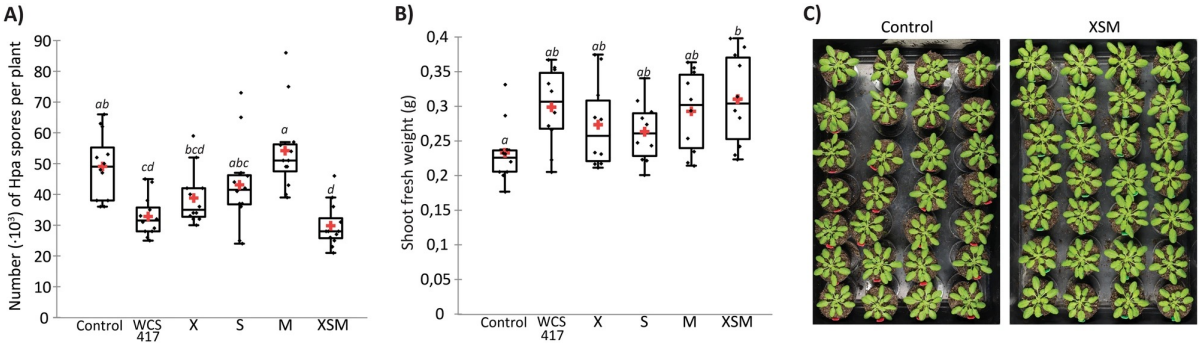
Modulation of the plant microbiome



44

Application of microbial consortia

Arabidopsis thaliana - Hyaloperonospora arabidopsidis

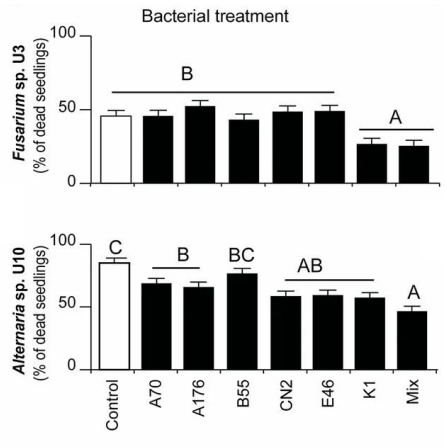


Berendsen et al. 2018

45

Application of microbial consortia

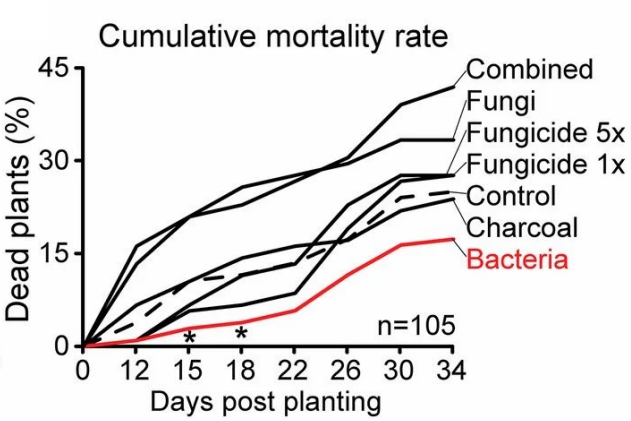
Nicotiana attenuata

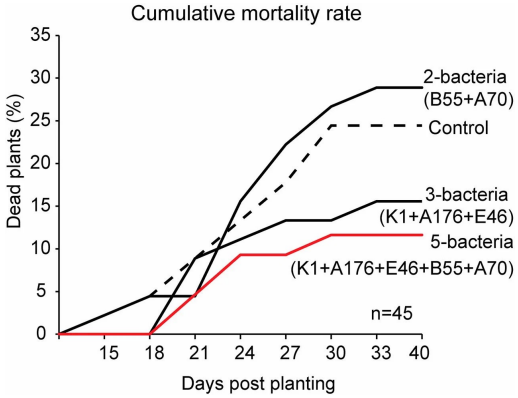


Santhanam et al. 2015

46

Application of microbial consortia



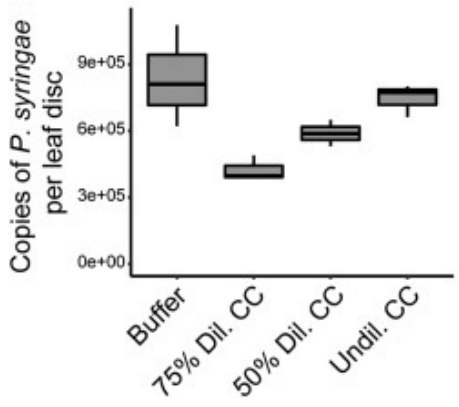


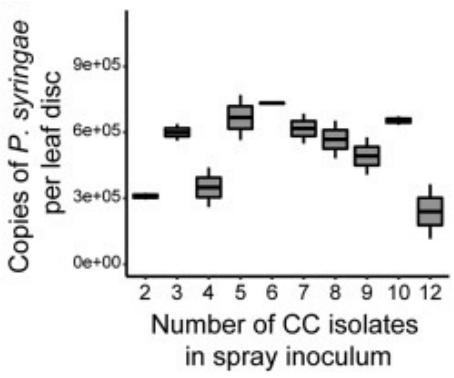
Santhanam et al. 2015

47

Application of microbial consortia

Solanum lycopersicum



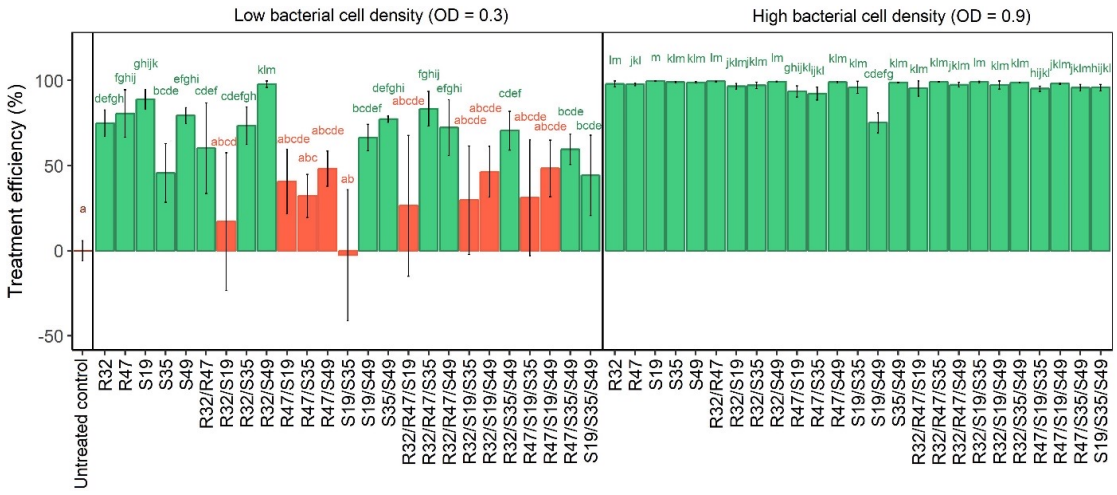


Berg et al. 2018

48

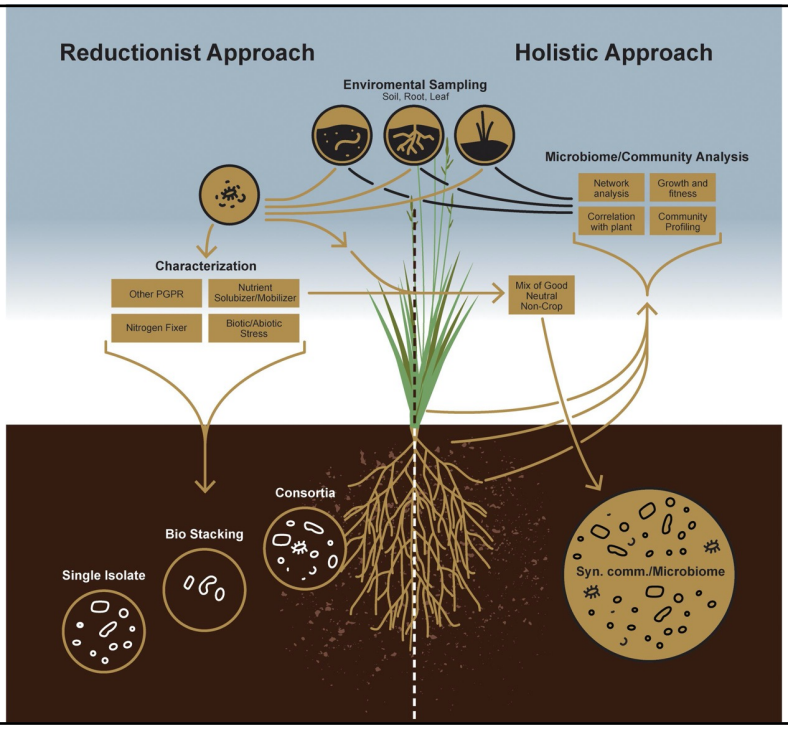
Application of microbial consortia

Potato-Associated *Pseudomonas* versus *Phytophthora infestans*



Vrieze et al. 2018

49



Ray et al. 2020

50