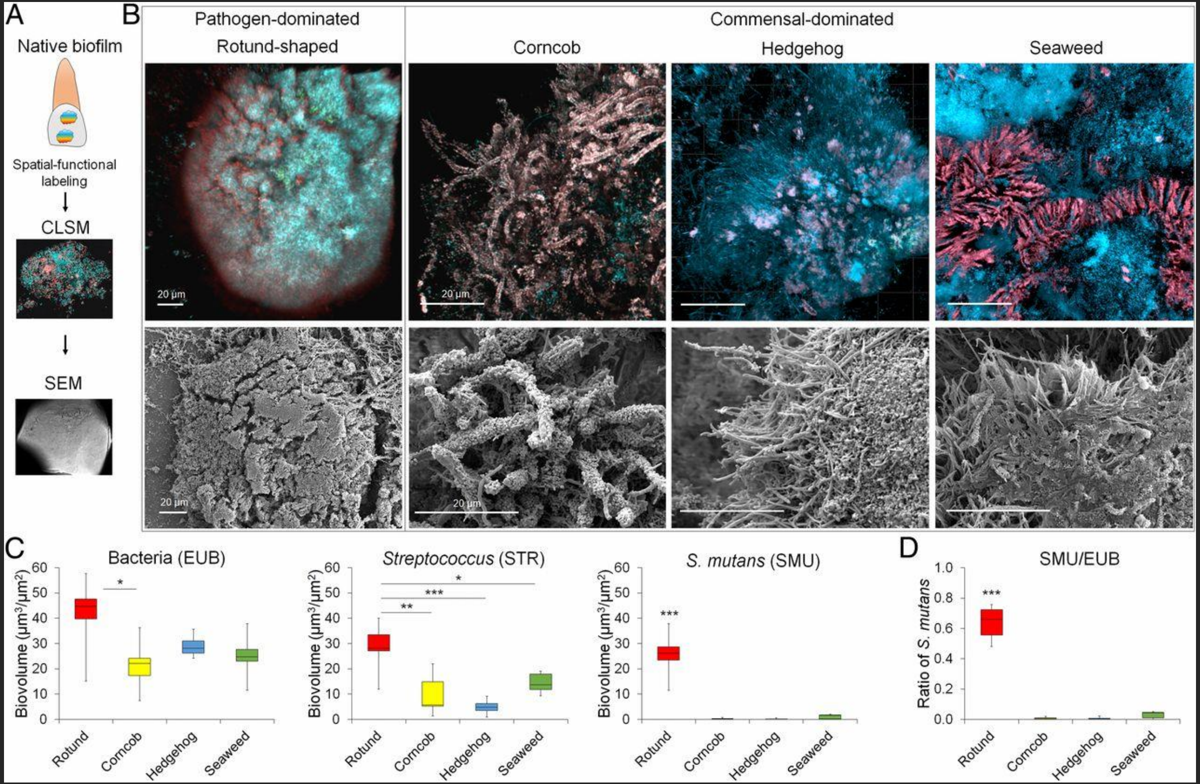
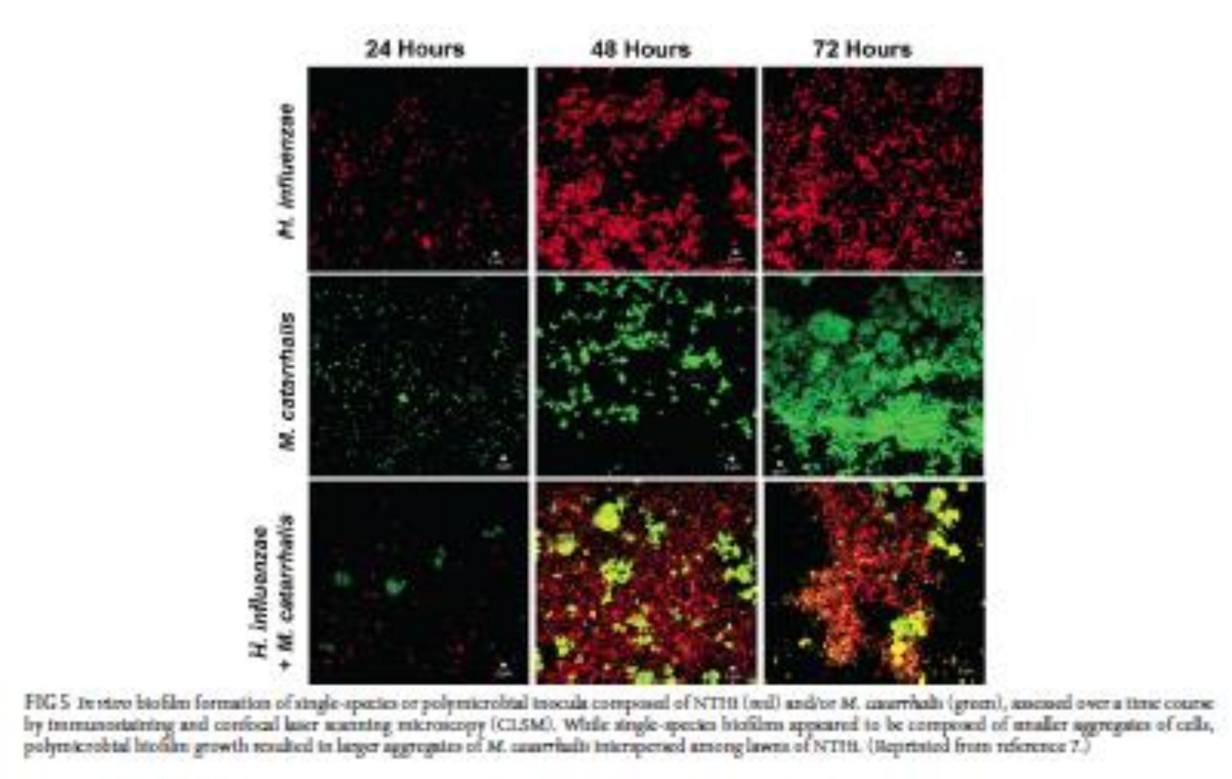
Inter- and Intraspecies interaction and communication

Most bacteria and fungi exist as part of larger polymicrobial communities in their natural settings



Dental caries

Kim et al. 2020 PNAS



Armbruster et al. 2010 mBio

Microbial behavior is modulated by neighboring organisms

Interspecies interactions can have profound and diverse consequences

E.g: i. modify virulence of pathogens

ii. alter antibiotic resistance profiles of mixed-species biofilms

iii. Increase or decrease microbial growth and biofilm formation

iv. increase or change production of specialized metabolites by fungi and bacteria

Intraspecies interactions are essential in survival strategies and mechanisms

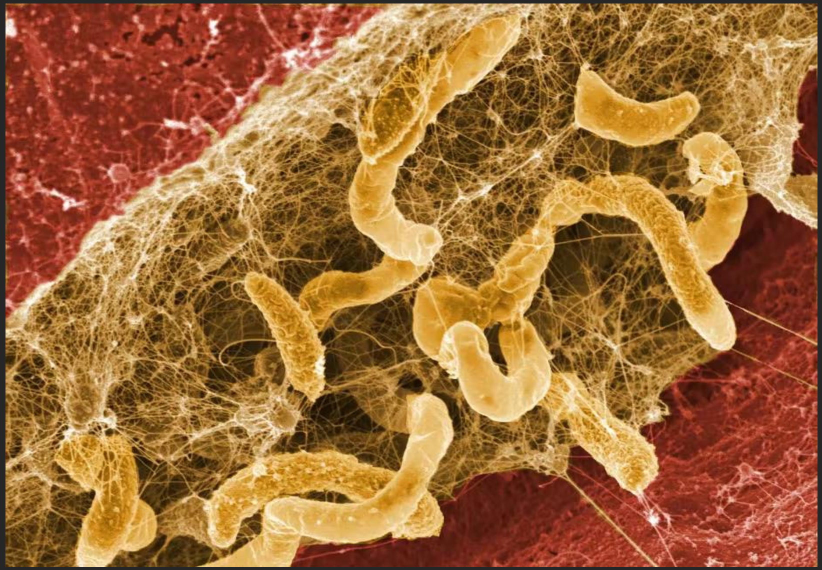
E.g: i. Bacterial communities develop in synchronized fashion to face changing environments

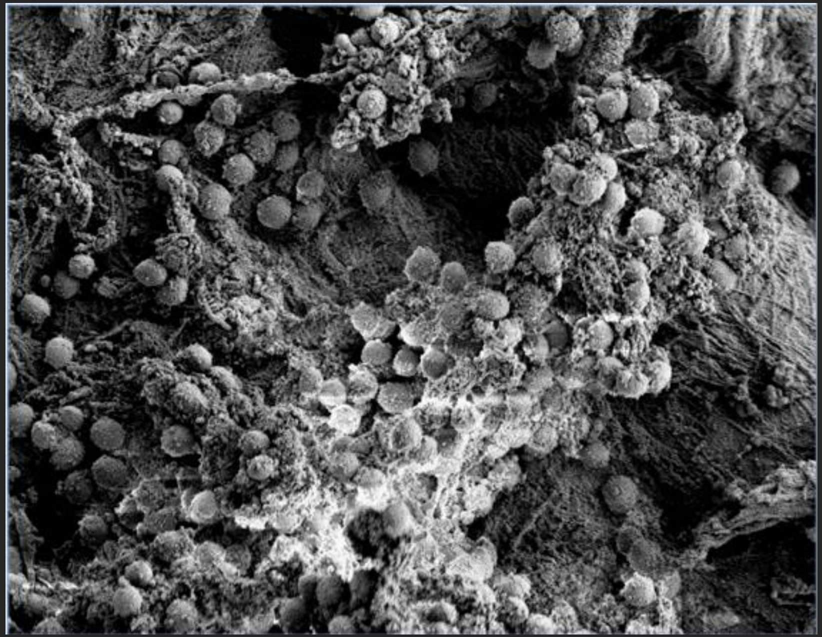
i. Biofilm formation, in which bacteria and fungi share nutrients and protection

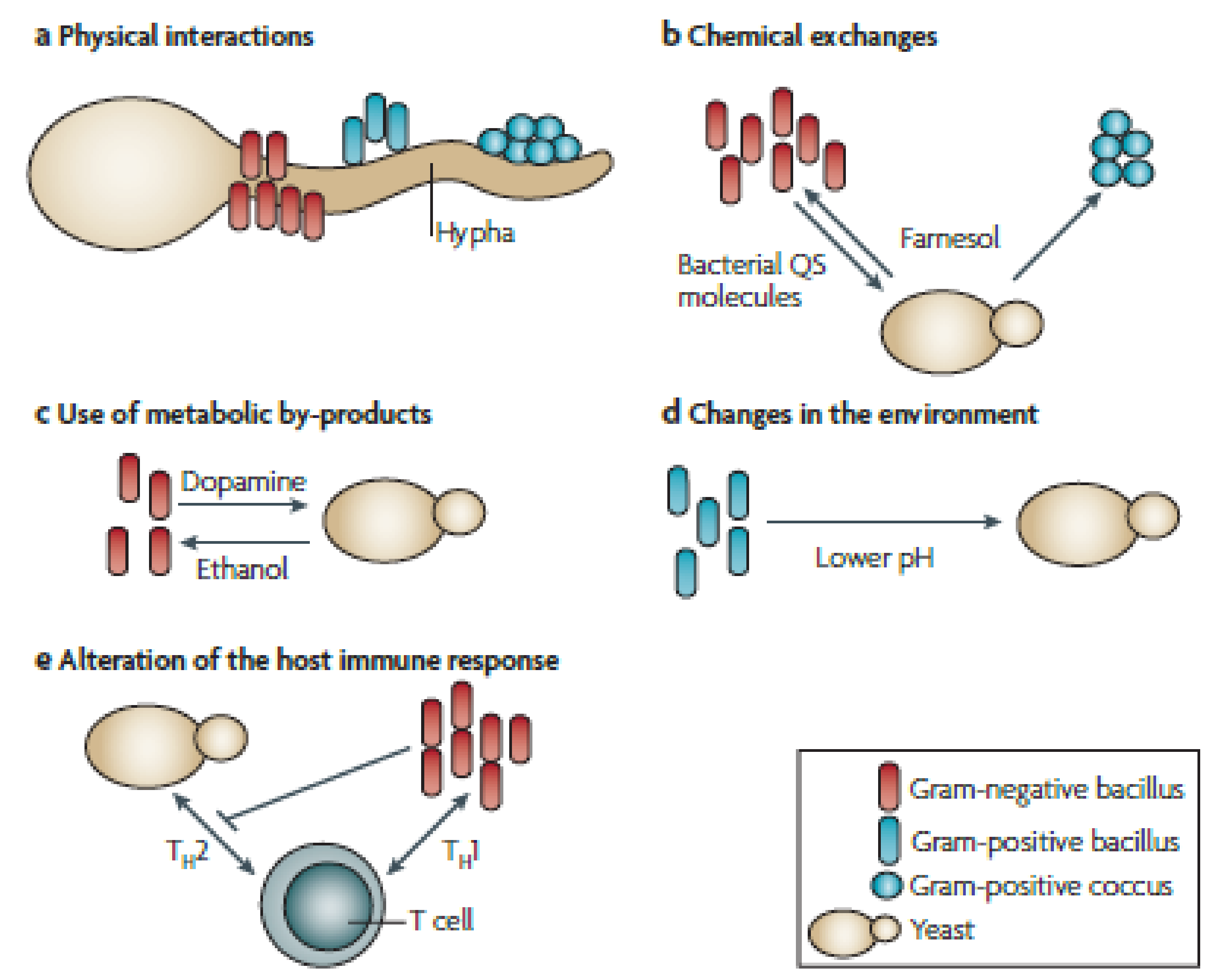
Communitarian behavior

Biofilm

- Syntrophic consortium of microorganisms (same or different species); cells stick to each other and often also to a surface
- Adherent cells become embedded within slimy extracellular matrix composed of extracellular polymeric substances (polysaccharides, proteins, lipids and DNA) - three-dimensional structure
- High resistance to antibiotics
- Quorum sensing plays an important role in regulating biofilm formation
- “Cities for microbes"







QS, quorum-sensing; TH, T helper cell

a | Bacterial attachment to fungal surface or co-aggregation with fungal cells, formation of bacterial biofilms on the surface of fungal hyphae and formation of mixed-species biofilms on abiotic or host surfaces

b | E.g., diverse bacteria produce small molecules that affect the morphology of *Candida albicans*, altering its ability to form biofilms or invade tissues. Farnesol (QS) secreted by *C. albicans* inhibits the synthesis of a pigment considered an important virulence factor in *Staphylococcus aureus*

c | Use of metabolic by-products

d | Changes in the environment

e | Alteration of the host immune response

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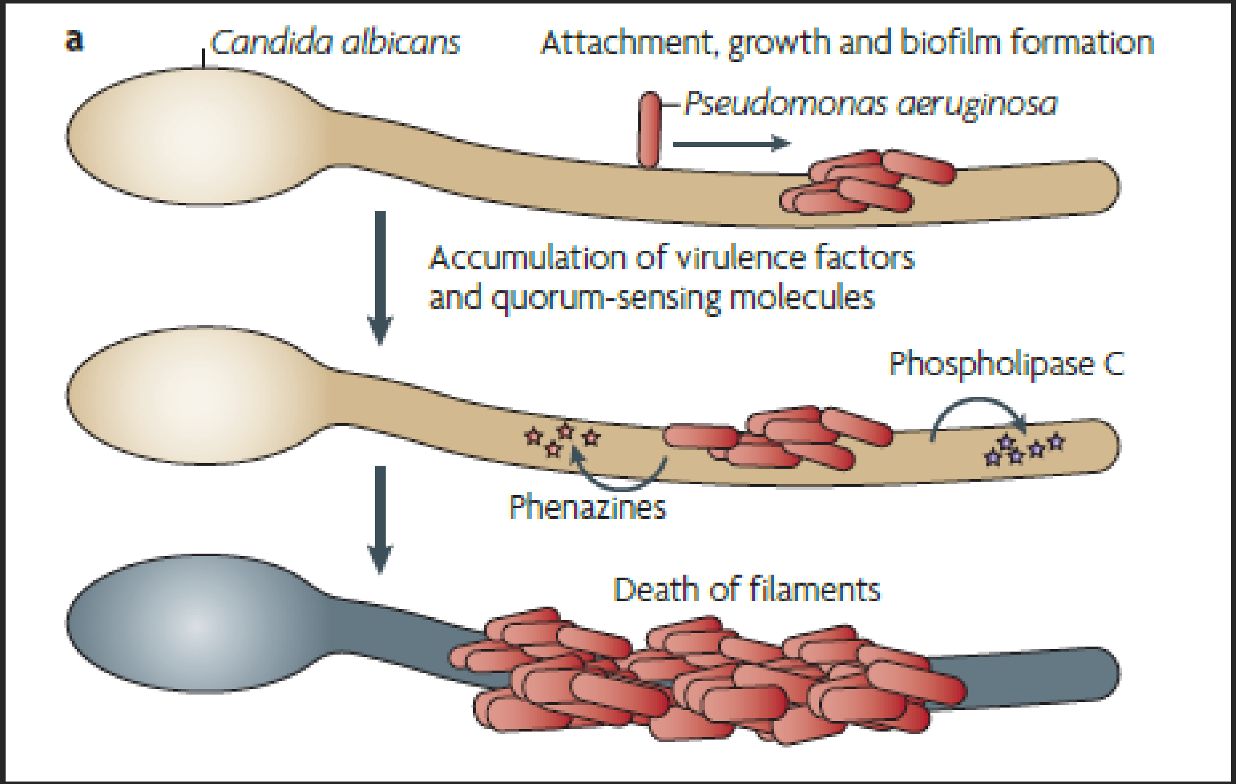


- bacterial secretion of anti-fungal molecules, transfer of toxins directly into fungal cells through secretion systems or nutrient depletion

Antagonistic

i. Antibiosis: Production of antibiotics or antifungal toxins by fungi and bacteria provide competitive advantage

ii. Physical interactions: bacterial aggregation or organized bacterial biofilms on the surface of fungal hyphae are associated with reduced fungal viability

iii. Environmental modifications: e.g., changes in pH can influence hyphae formation

Mutually beneficial interactions

Different species in mixed biofilm environments may protect each other against attacking immune responses or antimicrobial agents

COMMUNICATION

Signals from biotic or abiotic inputs are received by sensory organs and lead to sensory centers in the organism where appropriate behaviour is generated after internal interpretation according to available background experiences (memory)

Sign-mediated interactions

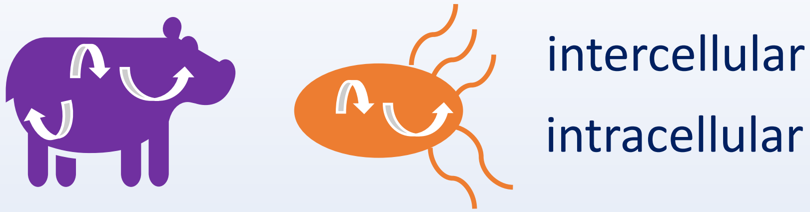
Precondition for all cooperation and coordination between at least two biological agents - cells, tissues, organs and organisms

essential for symbiotic interactions

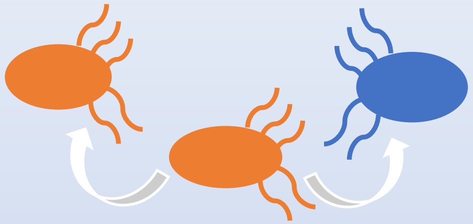
Signs are chemical molecules in most cases

Communication processes occur…

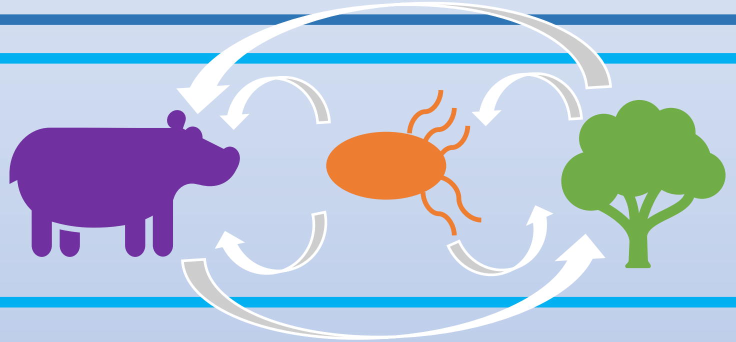
Within organisms – **Intraorganismic**



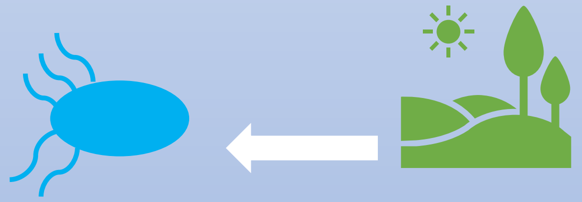
Between the same or related species that share a species-specific repertoire of rules and signs - **Interorganismic**



Between non-related species - **transorganismic**



Interpretation of abiotic influences as indicators to generate an appropriate adaptive behaviour



Quorum sensing

- Sign-mediated
- Chemical molecules are produced and secreted by bacteria or fungi
- Signs are recognized by the community and, dependent on a critical concentration and in a special ratio, control the population density
- These molecules trigger the expression of a great variety of gene transcriptions
- Many bacteria use multiple quorum sensing codes and each may be modulated by post-transcriptional or other regulatory engineering

Quorum Sensing

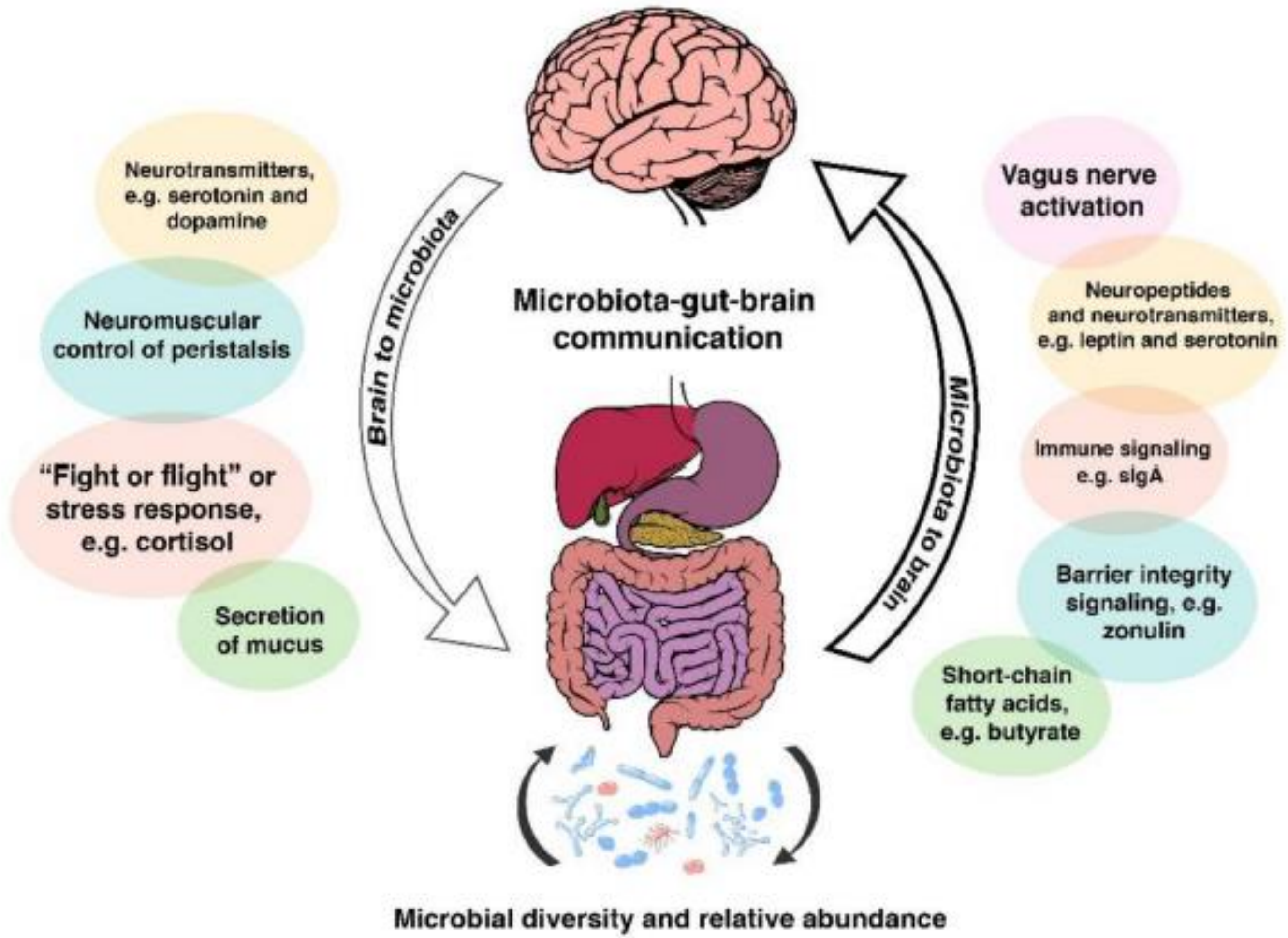
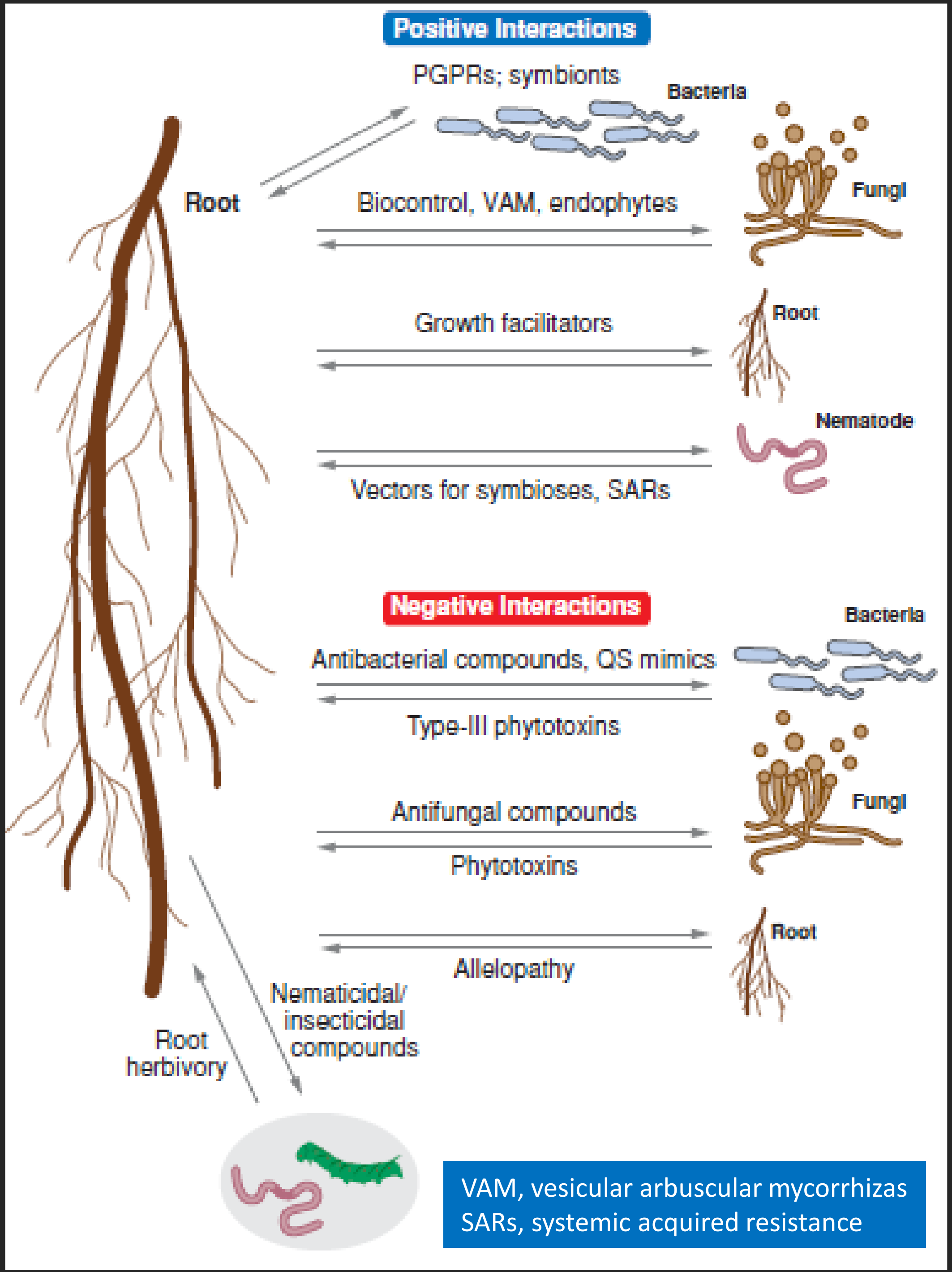


Figure 1. A simplified diagrammatic illustration of the bidirectional communication pathways between the gut microbiota and the brain, featuring a range of molecules originating in the gut that are involved in the upstream part of the communication system. Also featured is the "fight or flight" response, driven by the activation of the sympathetic nervous system, and how exposure of to that response plays an important role in the dysregulation of the intestinal ecosystem.



Plant-Plant

i. Excretion of phytotoxins - allelopathy
ii. Root exudates induce defense responses in neighboring plants: reduce susceptibility to pathogens; induce production and release of volatiles that attract predators of plant enemies
iii. Increase or decrease soil nutrient availability by altering soil chemistry and soil biological processes.

Plant-Microbe

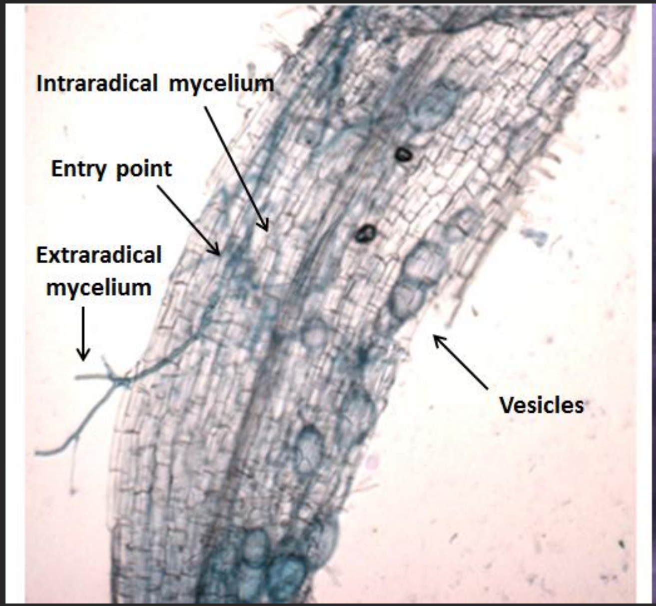
i. Rhizobia-nodulation: flavonoids/Nod factors
ii. AMF: strigolactones/Myc factors
iii. PGPR: chemotaxis
iv. PGPR: phytostimulators (auxins, citokinins, gibberellins); suppressive soils
v. Excretion of antimicrobials
vi. Quorum-sensing mimics

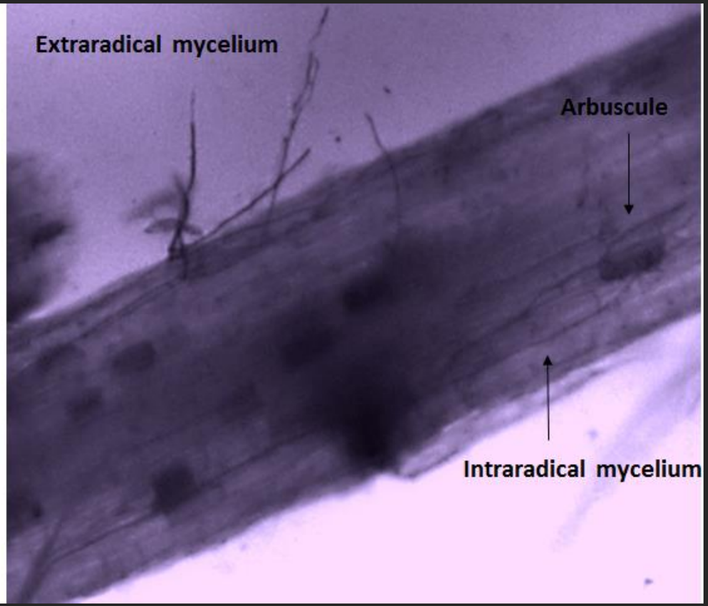
Plant-Animal

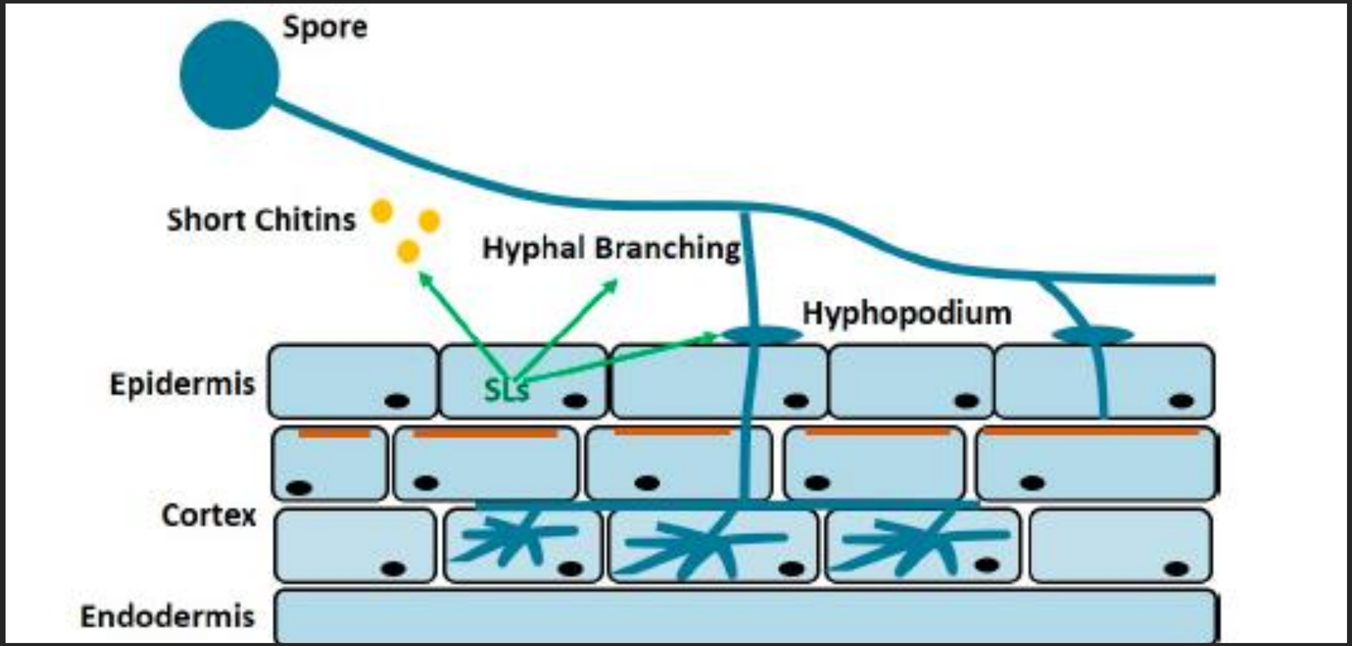
i. Nematicidal and insecticidal compounds
ii. Nematodes as symbiotic vectors

Ectomycorrhiza

Arbuscular mycorrhiza - AM







Multipartite Interactions

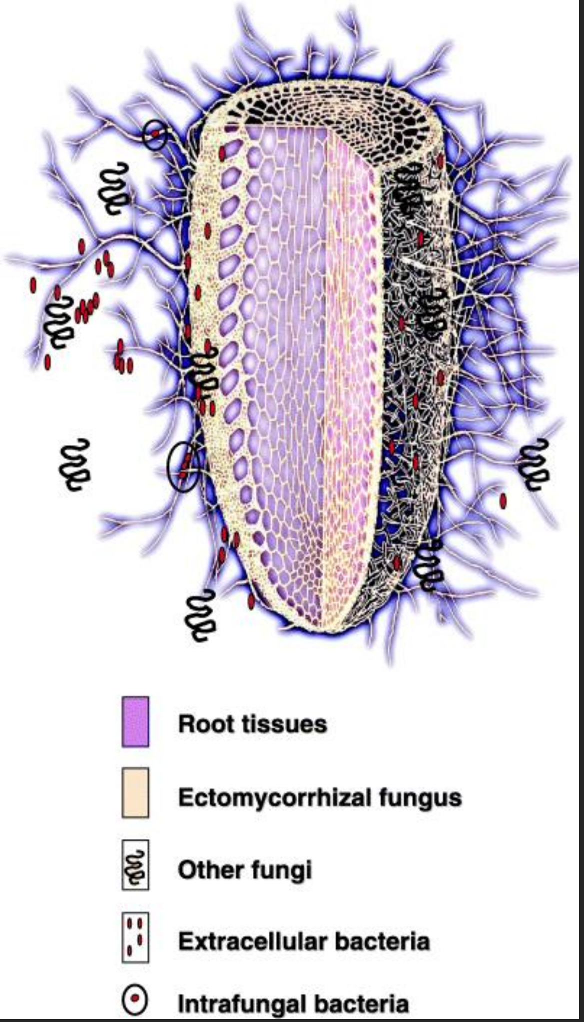
Bacteria-Fungi

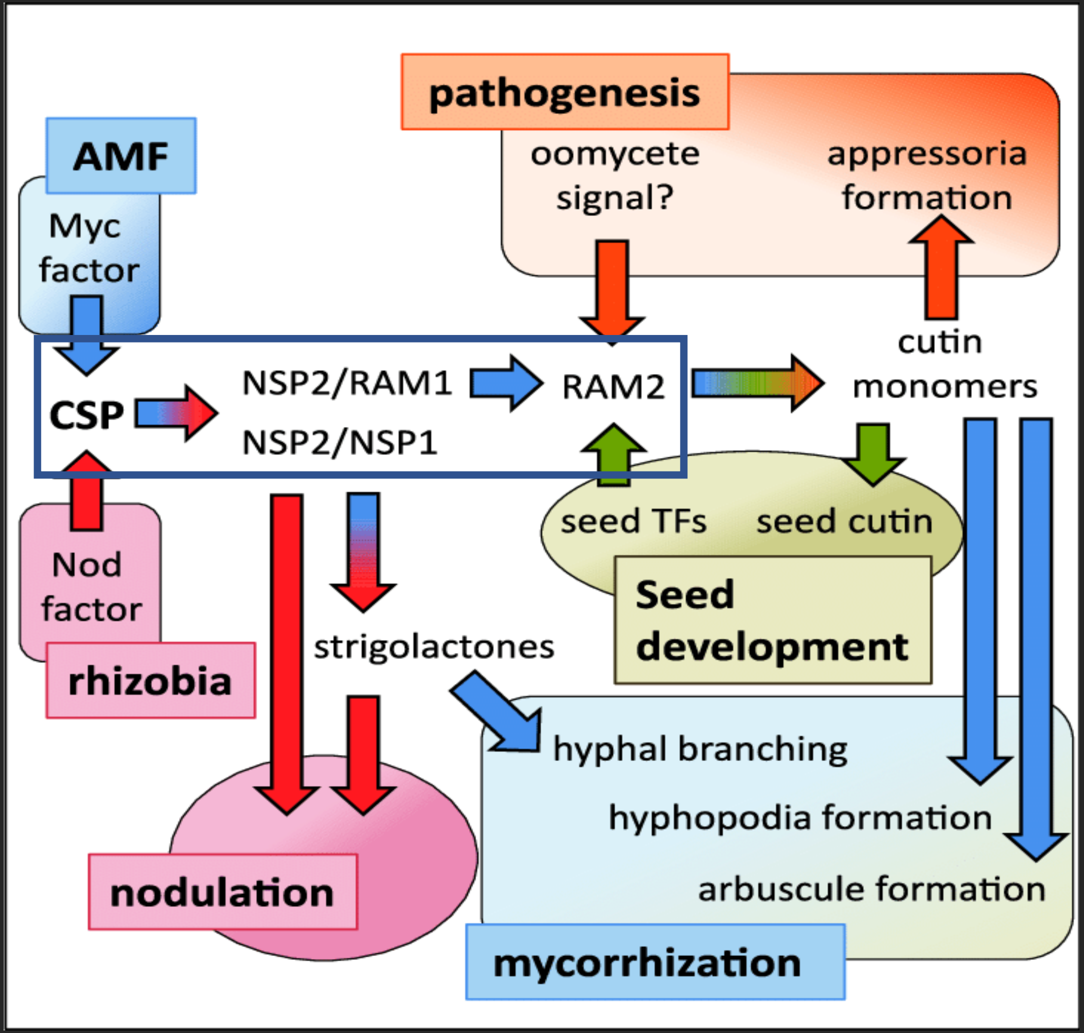
Plant-Bacteria

Fungi-Plant

And Animals!

Mycorrhiza helper bacteria





CSP: common signaling pathway

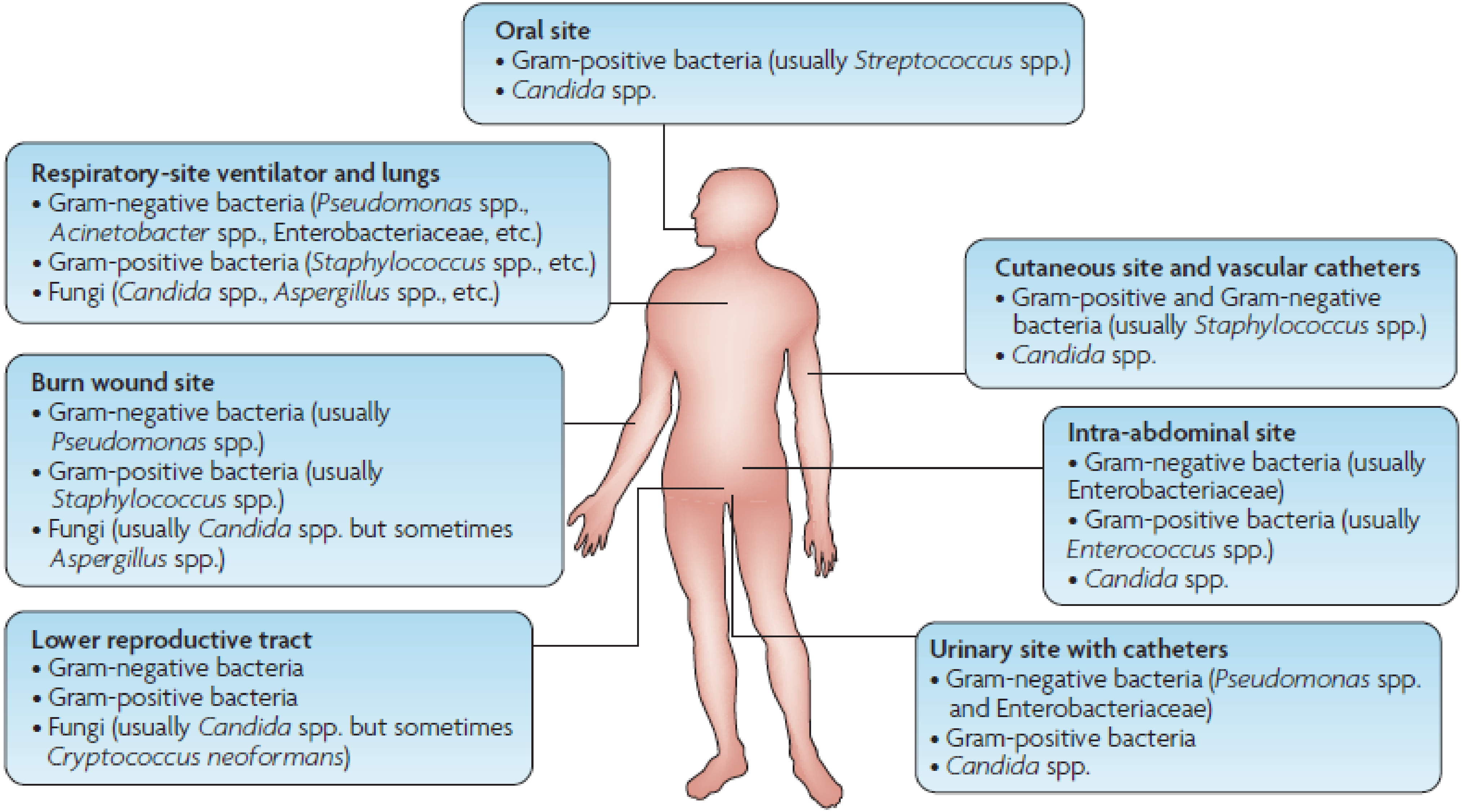


Figure 1 | **Clinically important sites for bacterial–fungal interactions.** Critically ill patients in the intensive-care unit are good examples of the diversity of sites at which bacteria and fungi can interact and cause disease. The boxes describe the organisms that are most commonly found at each site.

Microbial consortia

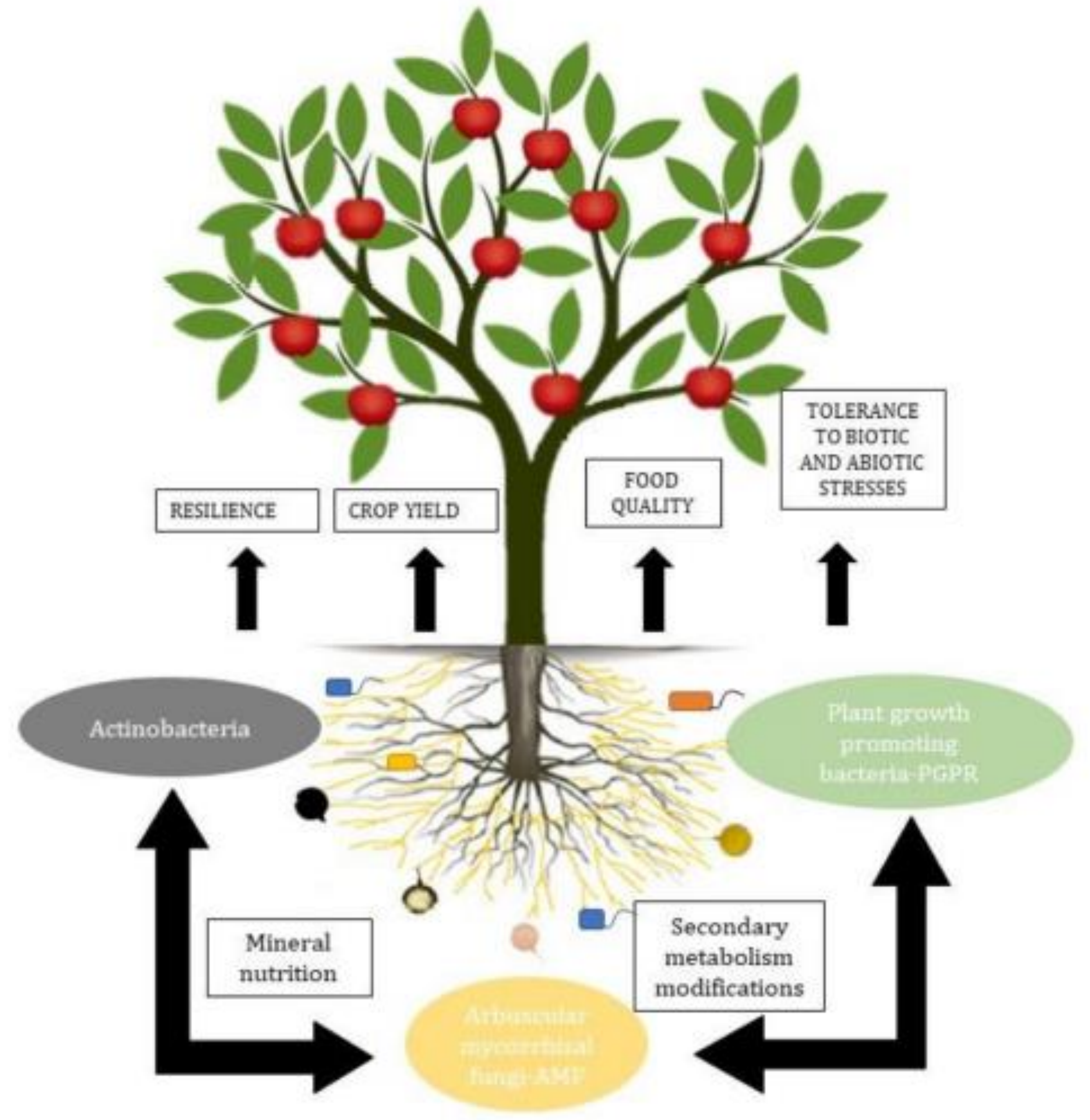
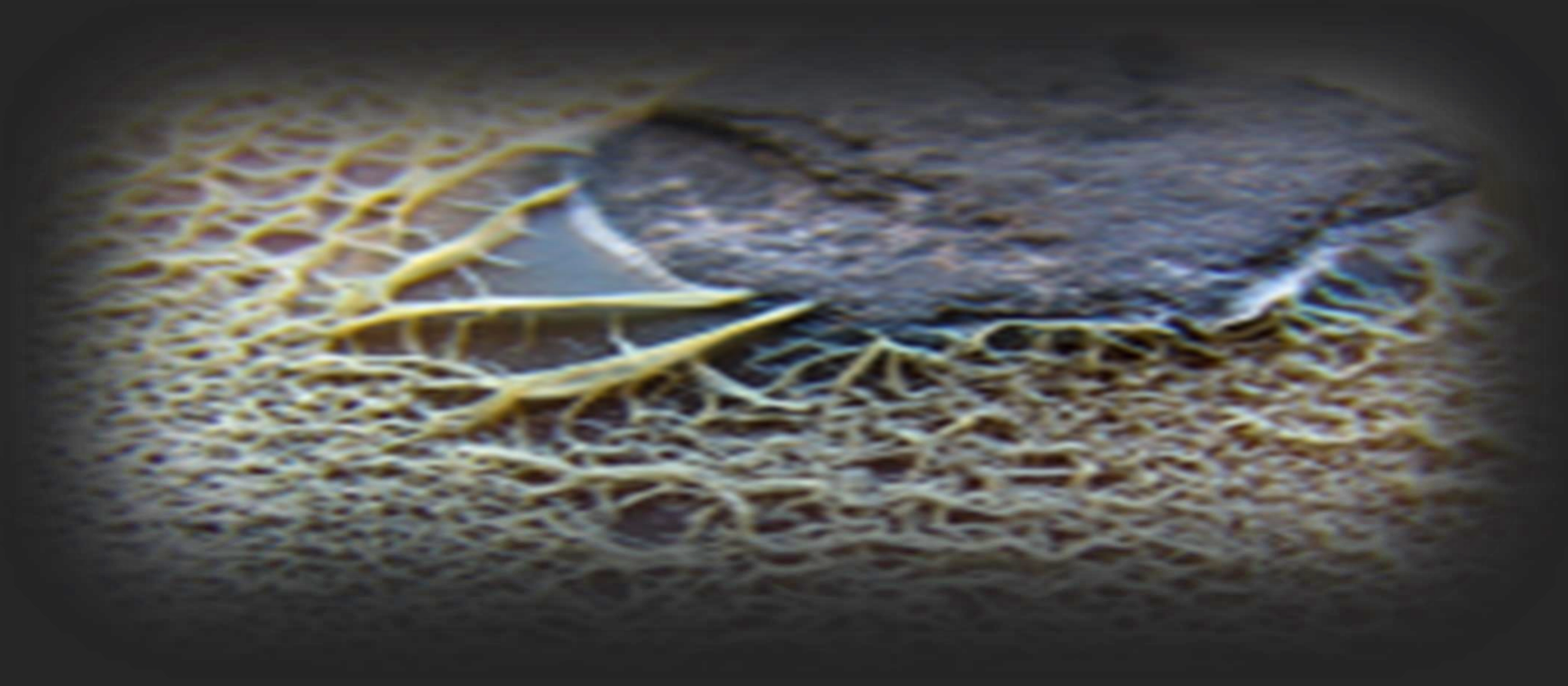
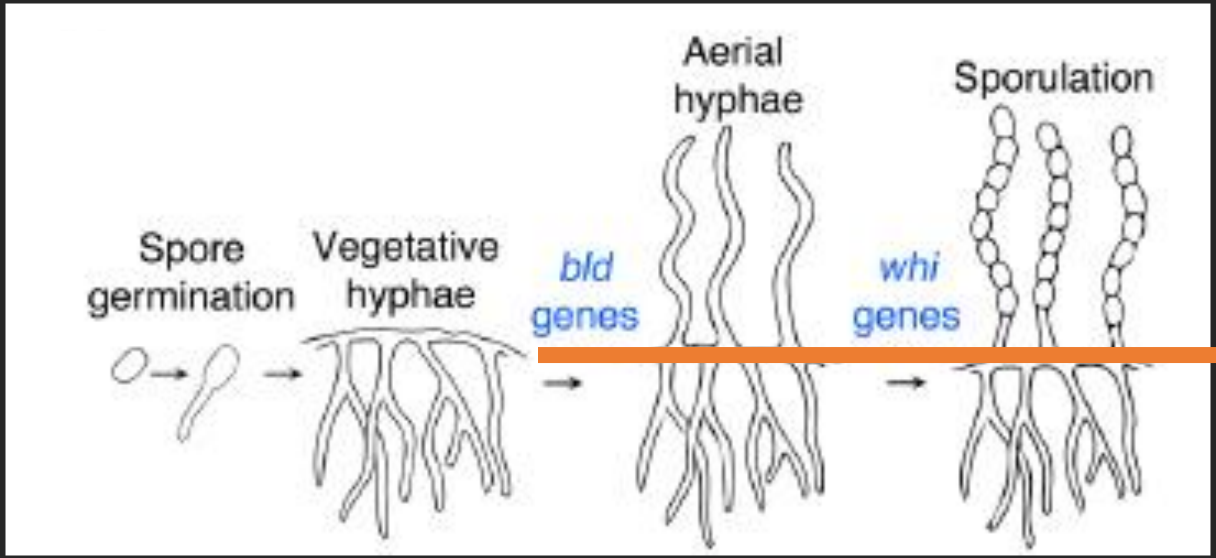


Figure 1. The ecosystem functions of microbial consortia of Actinobacteria, plant growth-promoting rhizobacteria (PGPR), and arbuscular mycorrhizal fungi (AMF) in sustainable agriculture.

Streptomyces exploration is triggered by fungal interactions and volatile signals

Jones *et al.* 2017 *Elife*





Life cycle of Streptomyces

Exploratory behaviour

Streptomyces venezuelae

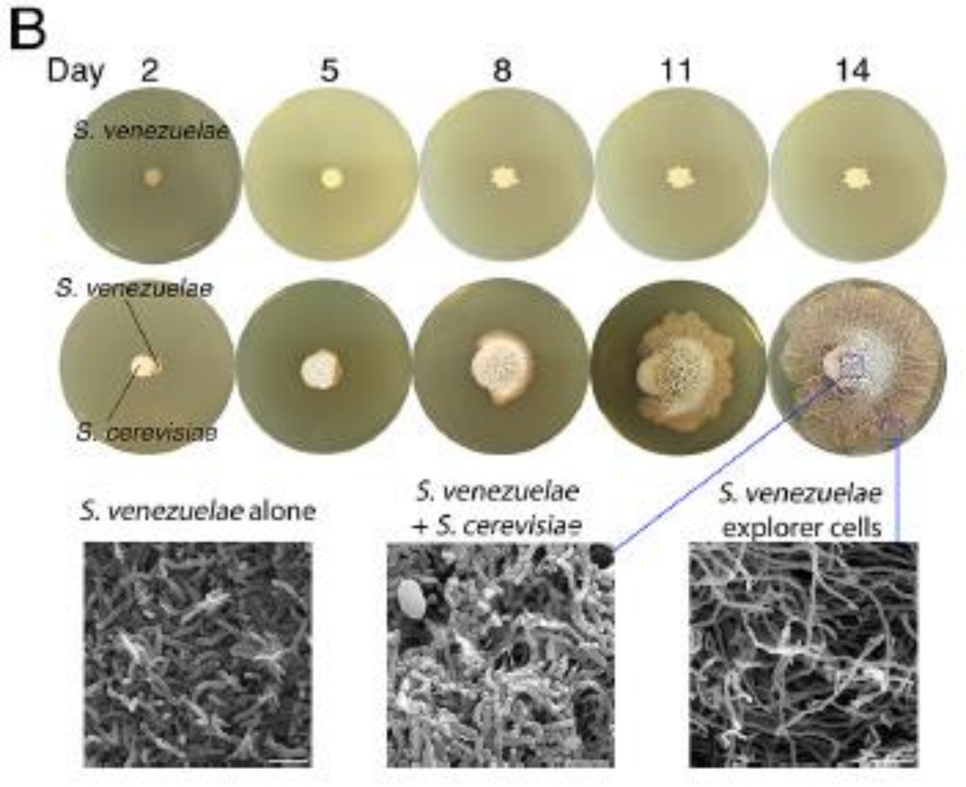
+

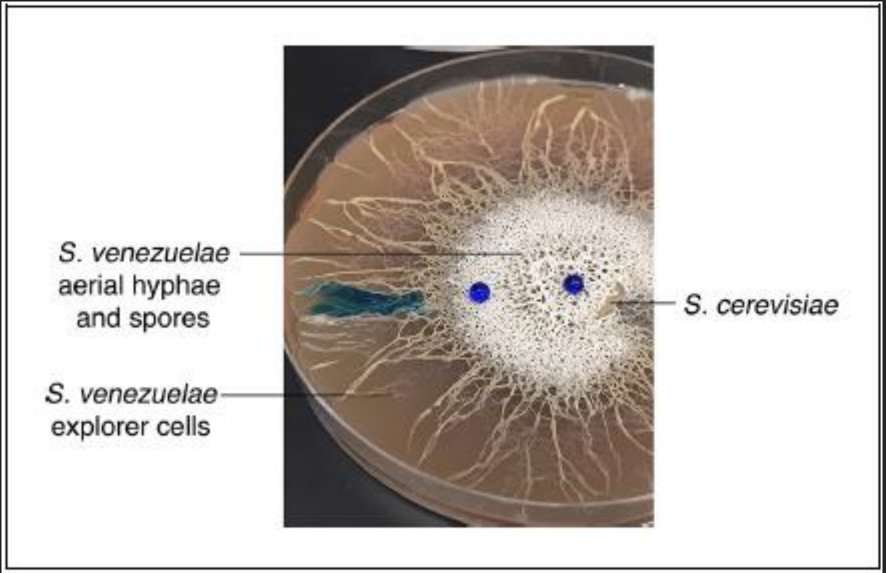
Sacharomyces cerevisae

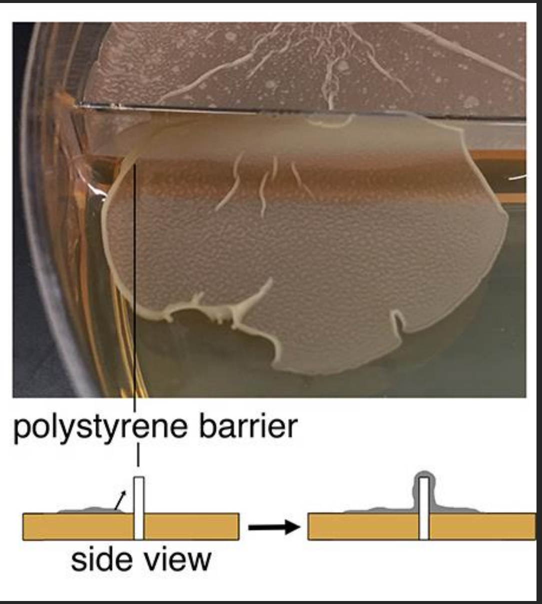
- Formation of non-branching aerial hyphae induced by signals that may be linked to nutrient depletion
- Aerial hyphae are hydrophobic

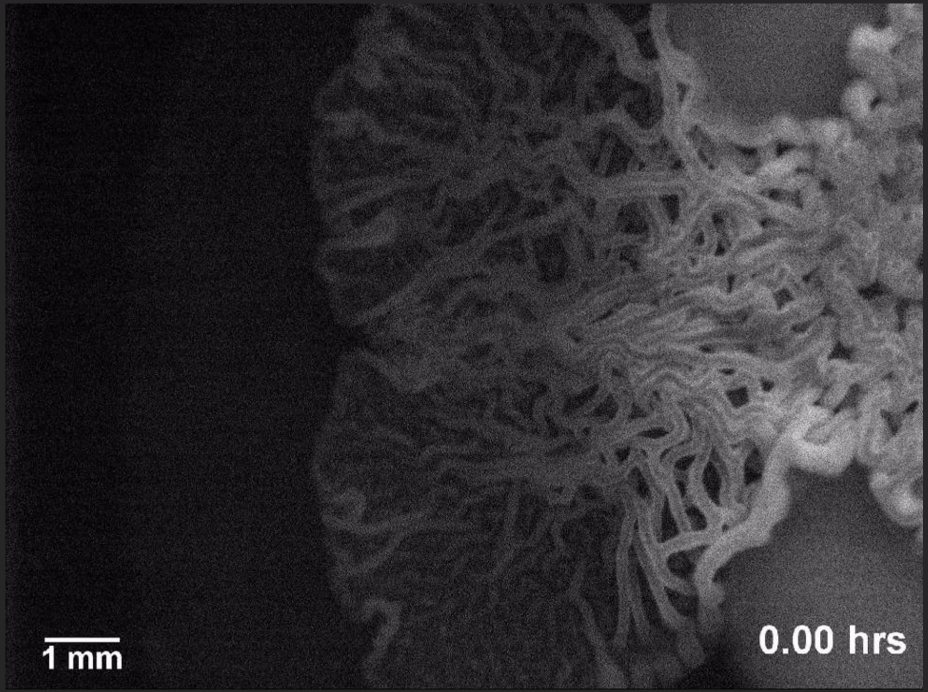
- Non-branching vegetative hyphal conformation that can rapidly traverse biotic and abiotic surfaces
- Hyphae are aerial, but hydrophilic

Physical association with yeast stimulates Streptomyces exploratory behaviour

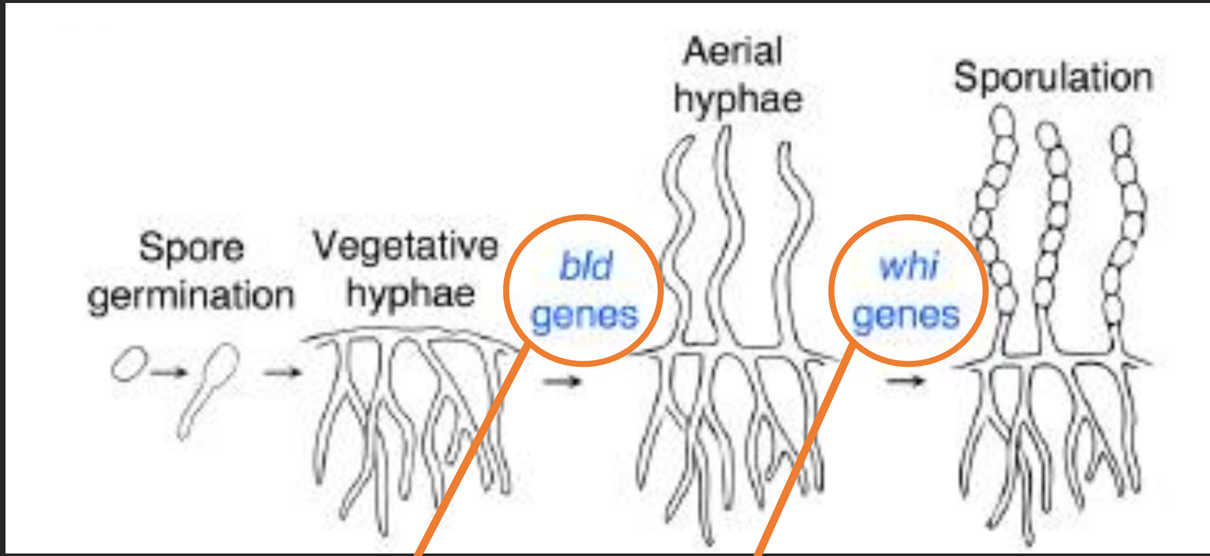







1 mm
0.00 hrs

1. Does exploratory growth require classic developmental regulators?



Life cycle of Streptomyces

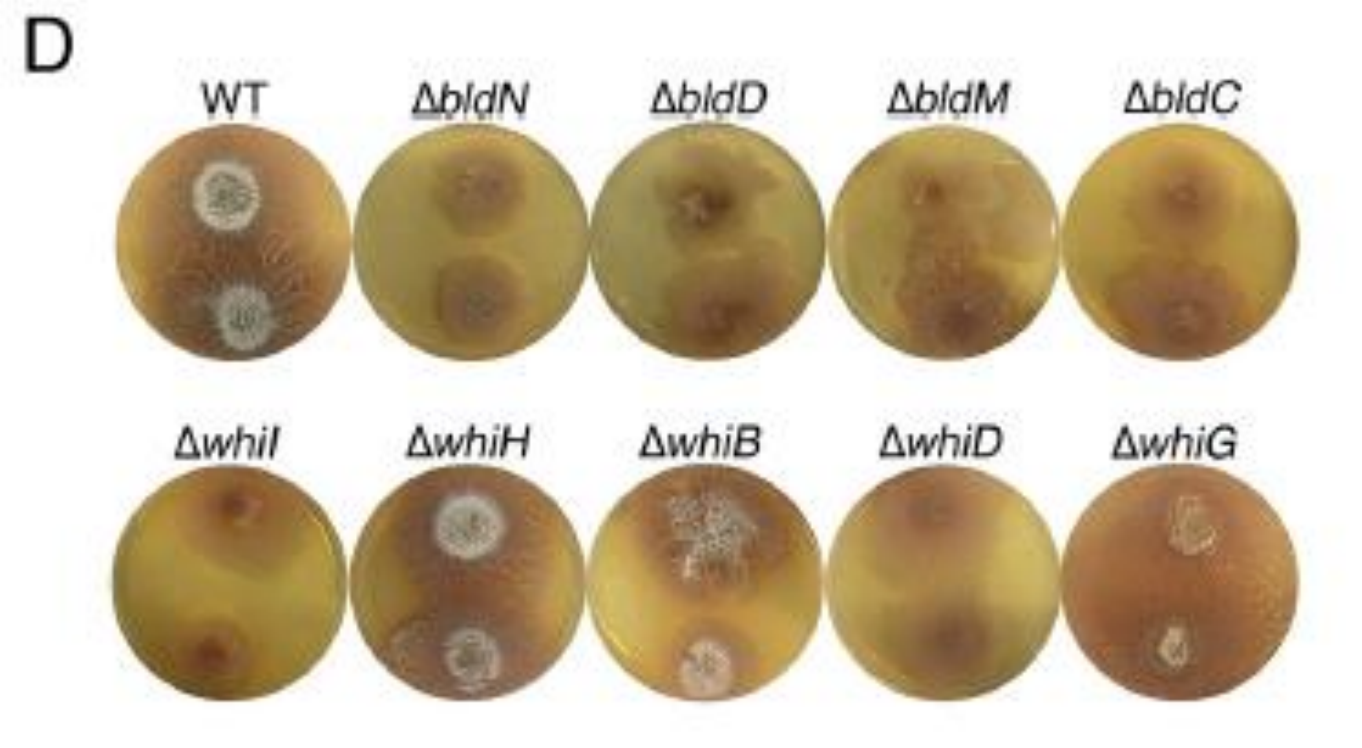
- ***bld*** required for transition from vegetative growth to aerial hyphae formation

  bld mutants fail to raise aerial hyphae

- *whi* required for differentiation of aerial hyphae into spore chains

  whi mutants fail to sporulate

1. Does exploratory growth require classic developmental regulators?



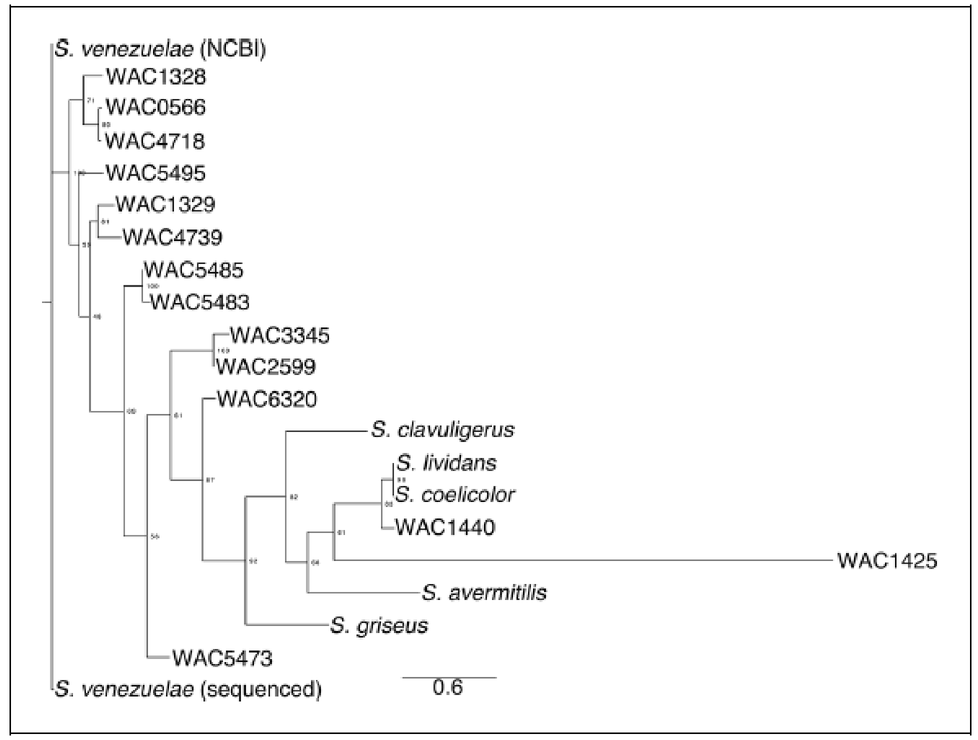
bld mutants fail to raise aerial hyphae
whi mutants fail to sporulate

All mutant strains displayed a similar exploratory behavior to the wild type after 14 days

Exploratory growth is distinct from the known *Streptomyces* life cycle: new form of growth

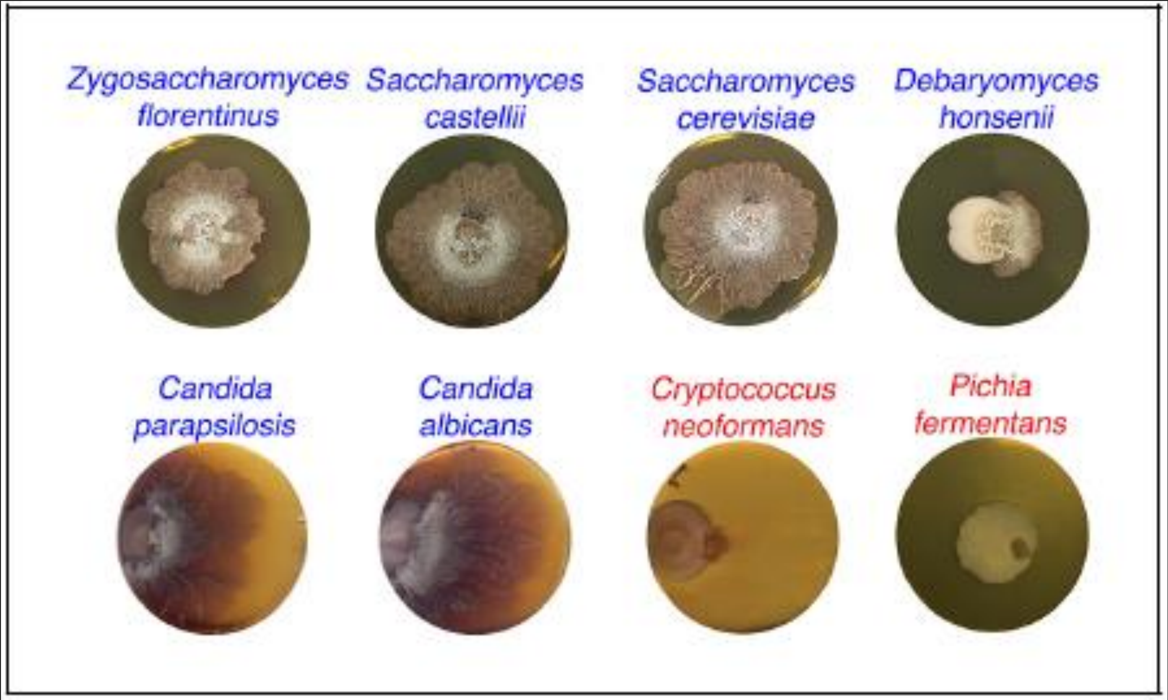
2. Is exploratory growth unique to *S. venezuelae*?

- *S. coelicolor, S. avermitilis, S. griseus* and *S. lividans* did not exhibit exploratory growth when plated next to *S. cerevisiae*
- 200 wild *Streptomyces* isolates: 19 strains (~10%) exhibited exploratory growth similar to *S. venezuelae*



Not a monophyletic group: exploratory growth is wide-spread in streptomycetes

3. Can exploratory behaviour be triggered by other fungi?



All species except *C. neoformans* and *P. fermentans*, induced exploratory behaviour

A broad range of microbial fungi could trigger exploratory growth.

4. The yeast TCA cycle must be intact to stimulate S. venezuelae exploratory behaviour

- 4309 *S. cerevisiae* knockout strains ⟶ 16 unable to promote exploratory behavior:

  13 mutations affecting mitochondrial function, including eight in genes coding for enzymes in the **tricarboxylic acid (TCA) cycle**

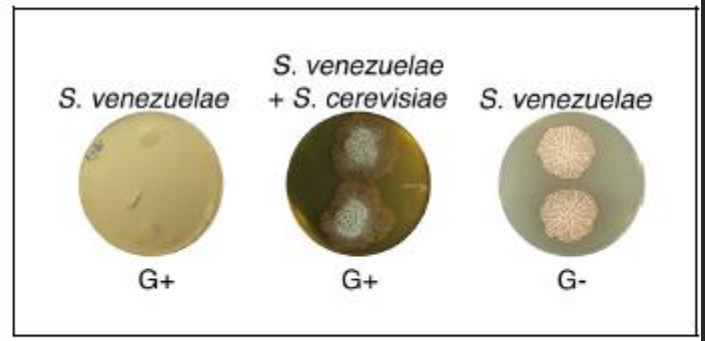
- *C. albicans* **LPD1** and **KGD2** mutants– **TCA cycle -** unable to promote exploratory behavior

↓

Fungal respiration, in particular TCA cycle, affects exploratory growth in *S. venezuelae*

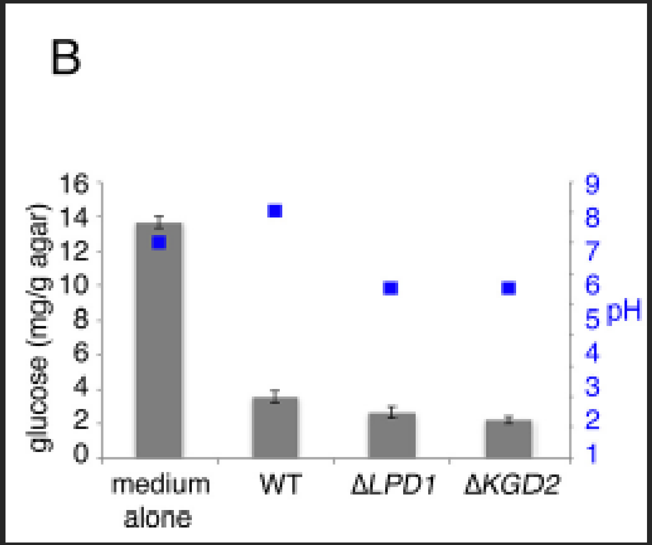
5. Is this effect linked to glucose uptake and/or consumption?

- *S. cerevisiae* consumes glu during growth on YPD agar → *S. venezuelae* exploratory growth may be triggered by glu depletion by yeast or a product of glu metabolism



→ **Glu represses exploratory behaviour**

BUT:

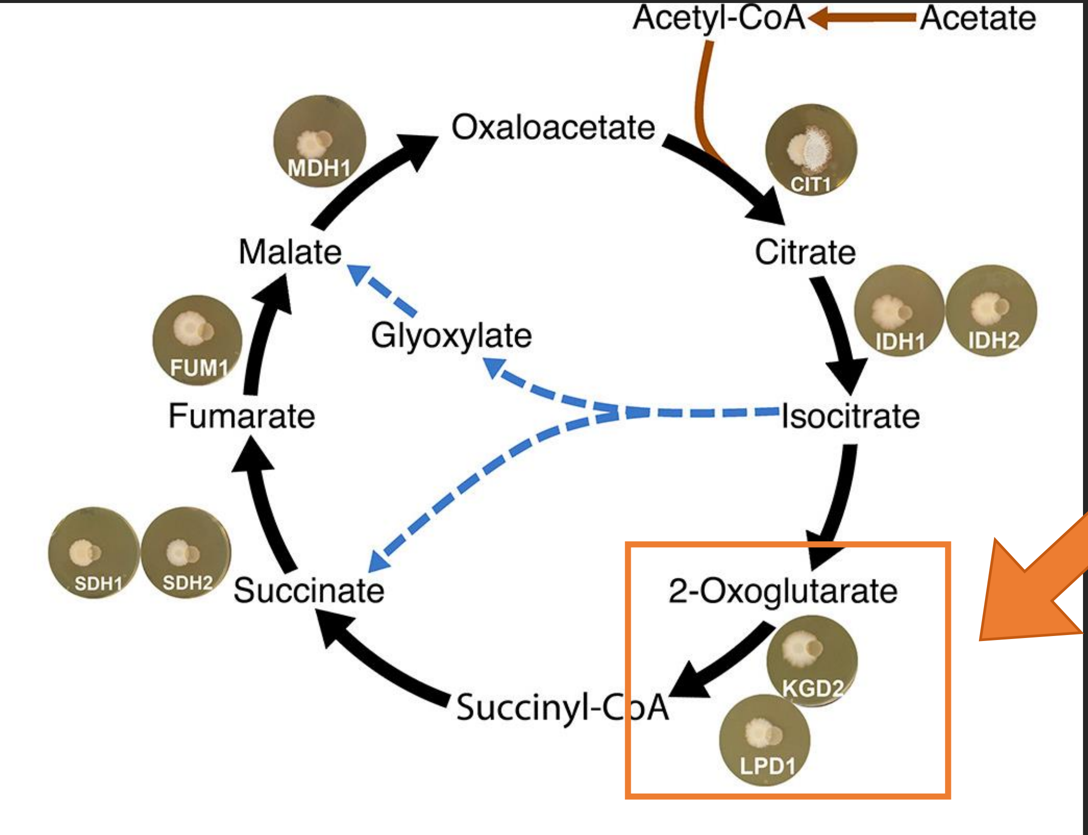


WT, DLPD1 and DKGD2 *S. cerevisiae* strains consumed similar levels of glu

↓

Other factors must be inhibiting *S. venezuelae* exploration when grown adjacent to these TCA cycle mutants

5. Is this effect linked to medium pH?

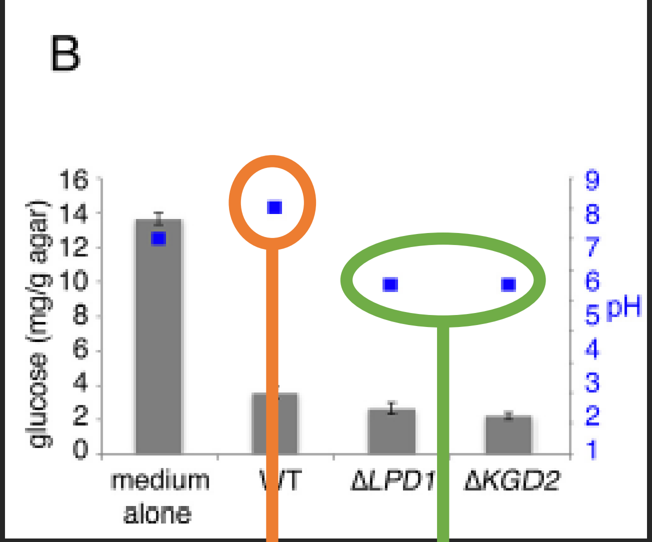


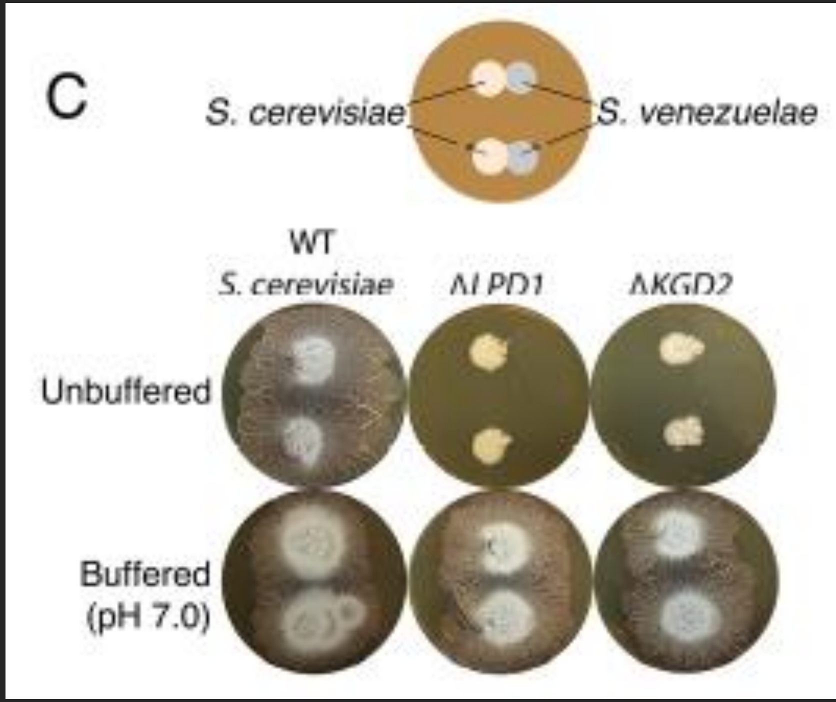
TCA cycle-associated *S. cerevisiae* mutants that failed to stimulate *S. venezuelae* exploratory behaviour were blocked after the production of citrate in the TCA cycle

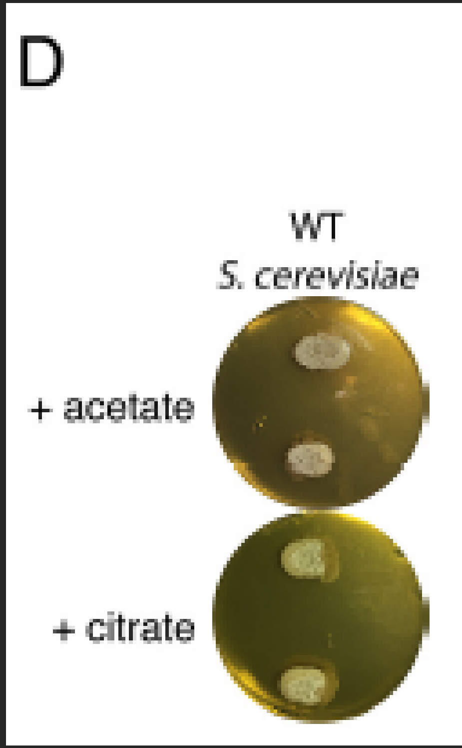
Hypothesis:

- TCA cycle disruption results in organic acid accumulation in *S. cerevisiae* mutants
  - Acids are secreted to maintain neutral intracellular pH
    - Acid secretion lowers medium pH

5. Is this effect linked to medium pH?

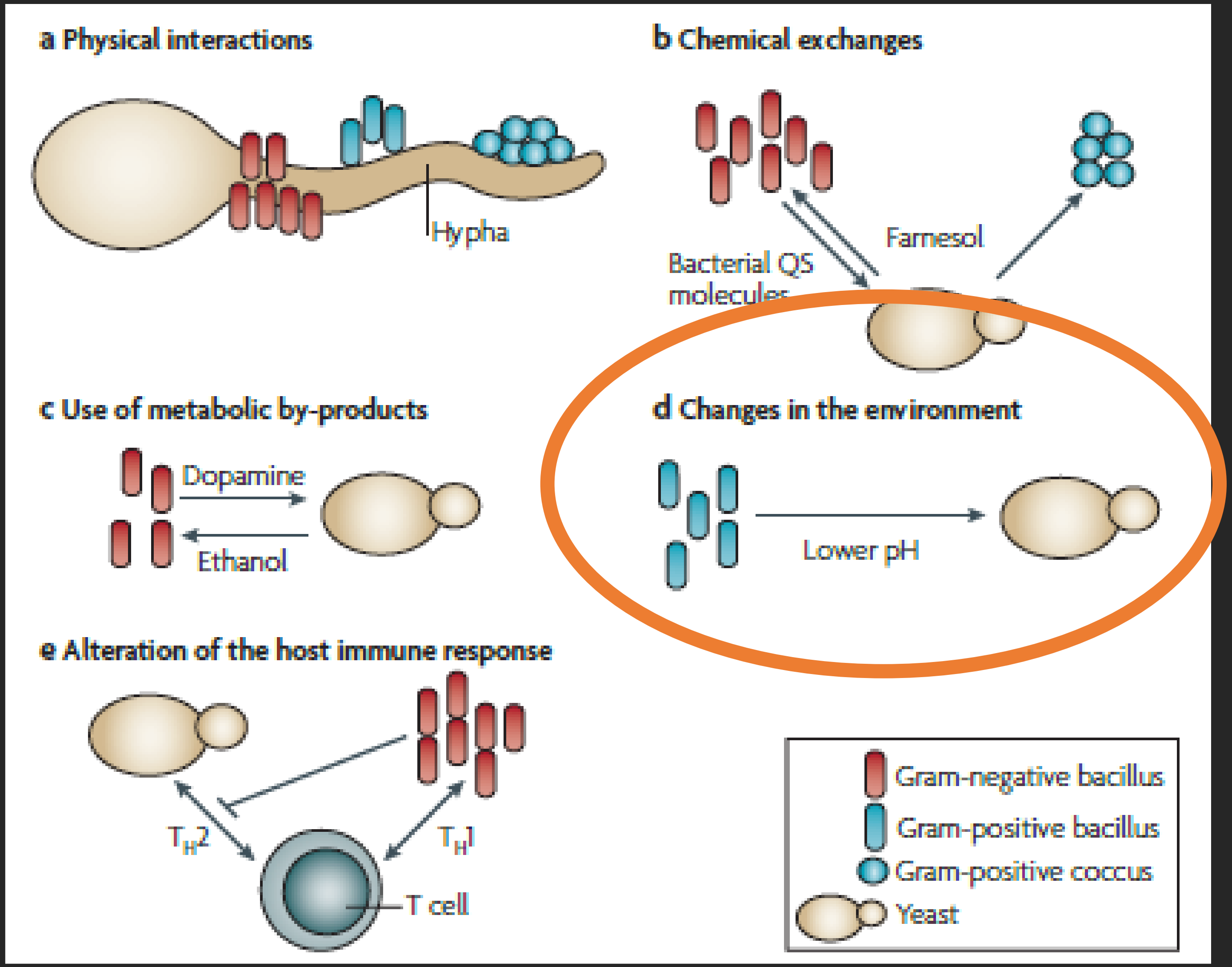






- WT raised agar pH to 7.5
- DLPD1 and DKGD2 lowered agar pH to 5.5

Exploratory behavior is pH dependent



QS, quorum-sensing; TH, T helper cell

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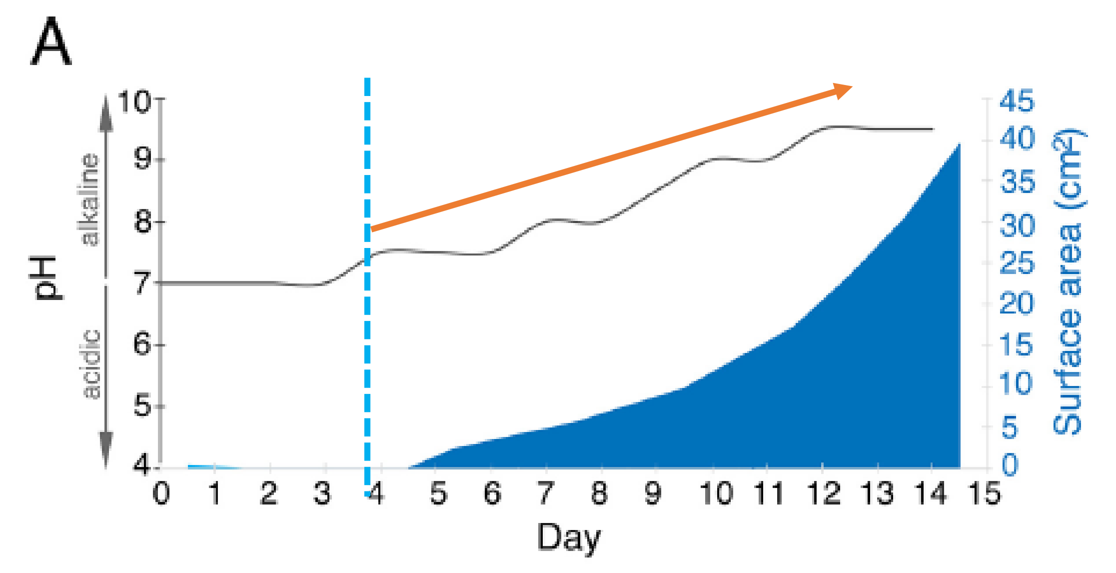
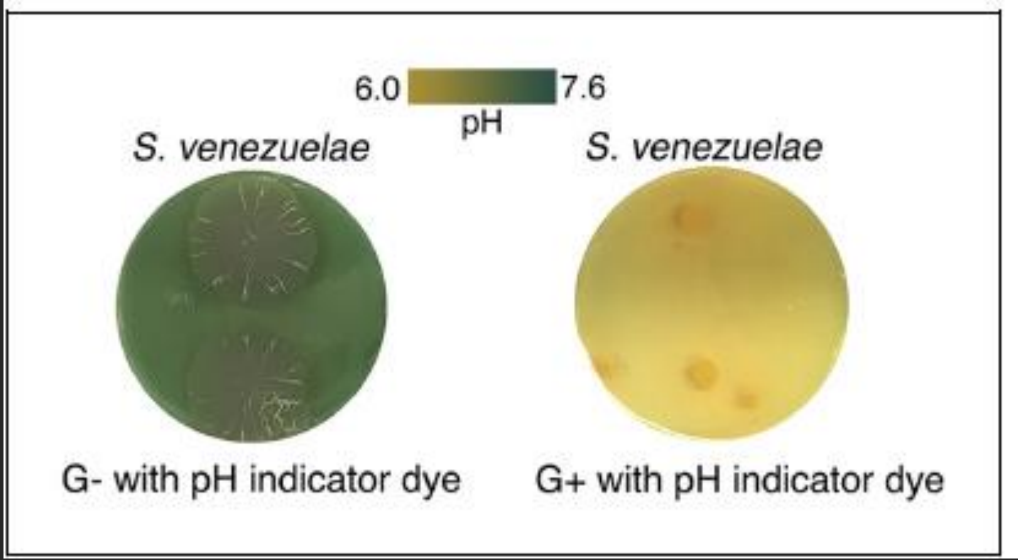


S. venezuelae exploration (with yeast) associated with pH increase



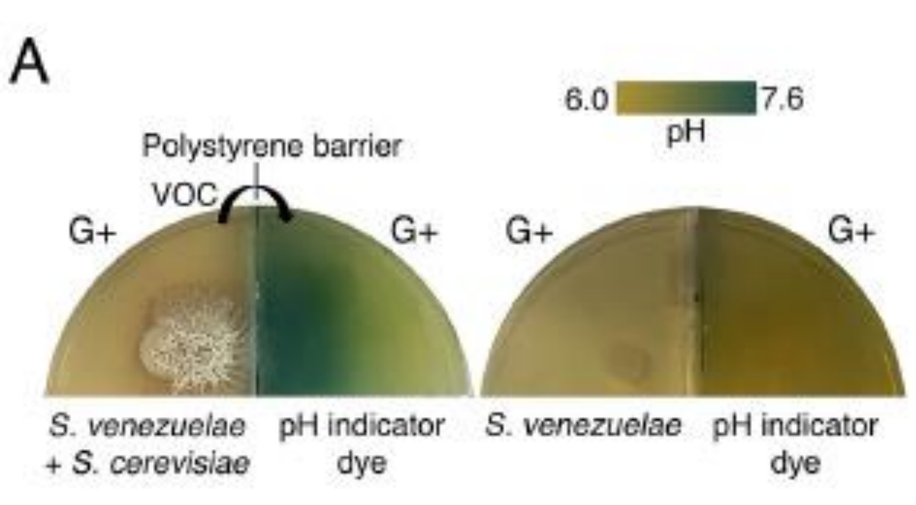
S. venezuelae grown on G- medium (in the absence of yeast) also increases pH

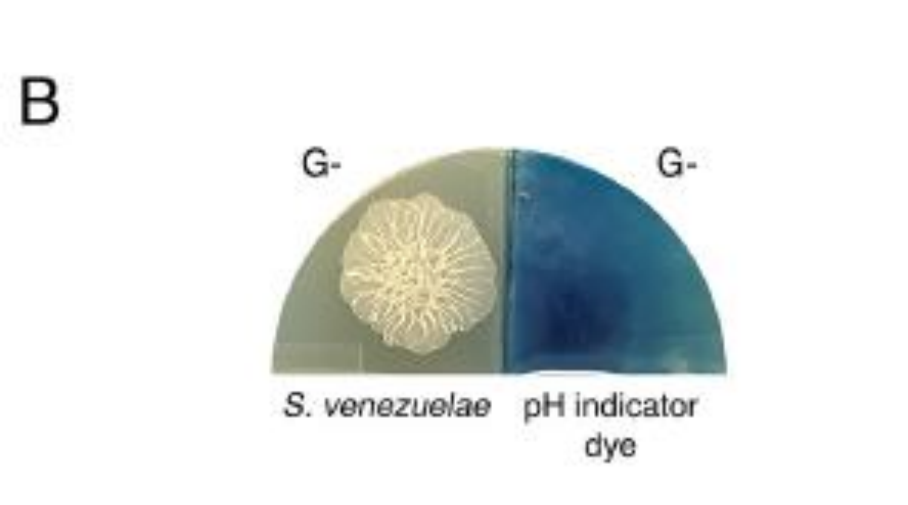
pH rise is promoted by Streptomyces

6. What increases pH?

secretion of diffusible basic compounds

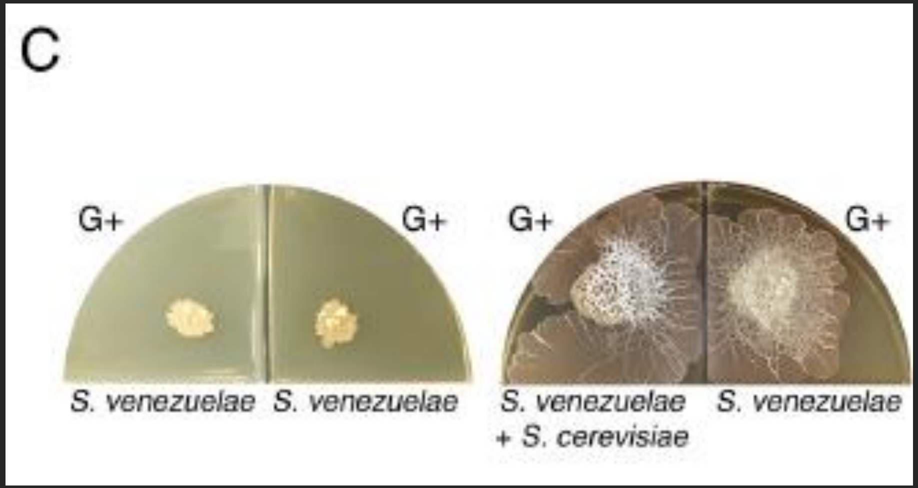
release of volatile organic compounds (VOCs)



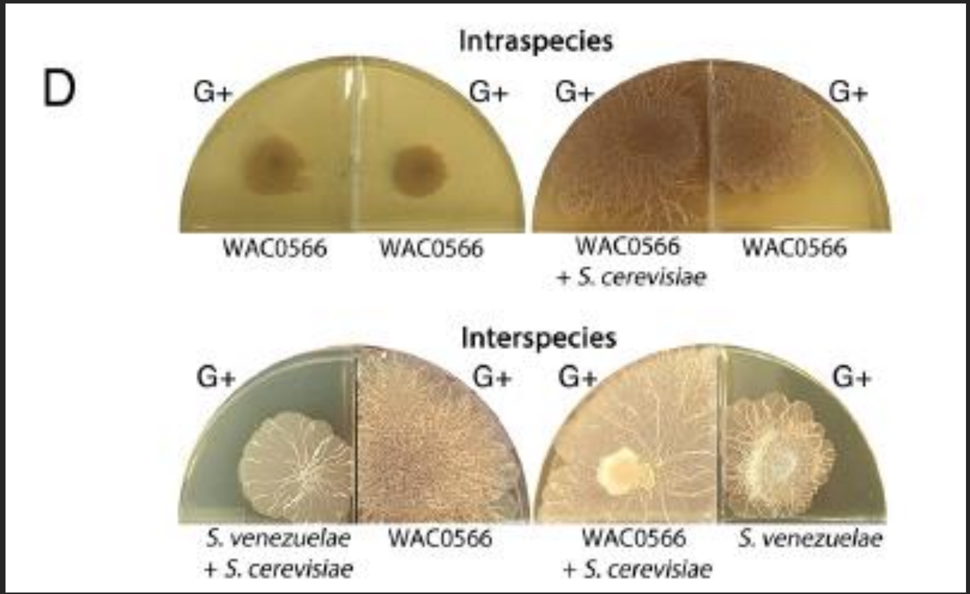


It's VOCs, released by *S. venezuelae*

6. What increases pH?



Streptomyces VOCs induce exploratory behavior in neighbouring Streptomyces



… of both the same and different species

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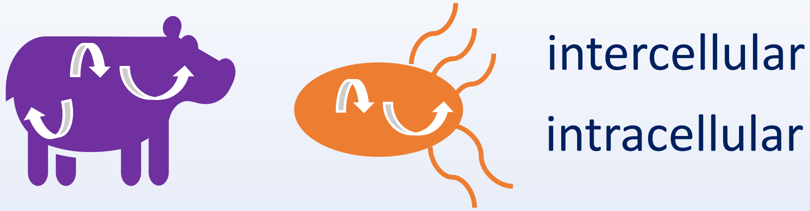
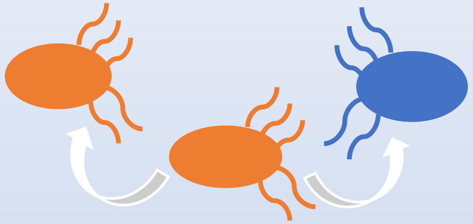
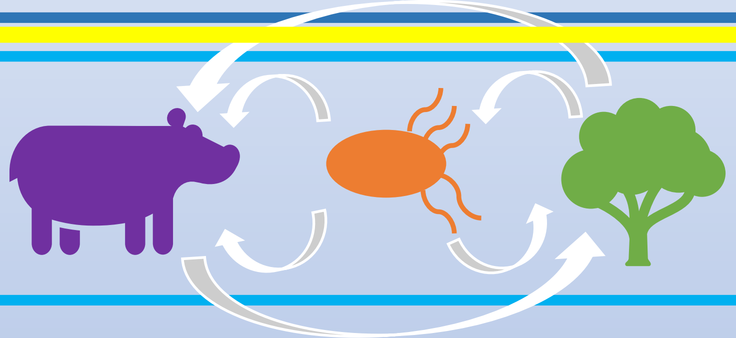
Within organisms – **Intraorganismic**



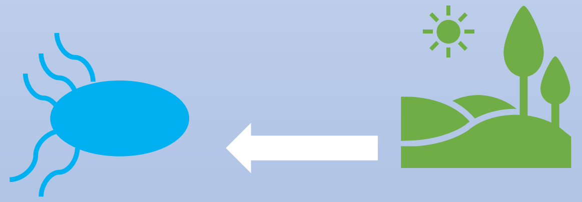
Between the same or related species that share a species-specific repertoire of rules and signs - **Interorganismic**



Between non-related species - **transorganismic**



Interpretation of abiotic influences as indicators to generate an appropriate adaptive behaviour

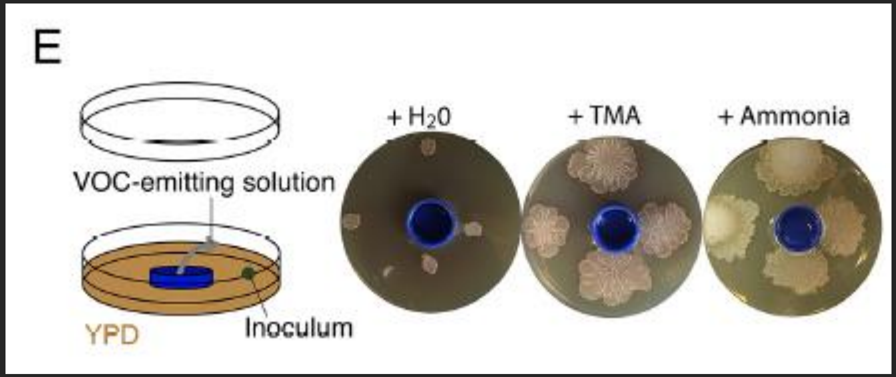


7. VOC identification and mechanism

Trimethylamine (TMA)

- Can raise the medium pH similarly to explorer cells…
- … inducing exploratory growth

i.e., TMA induces exploratory growth by increasing pH
(just like Streptomyces cultures)

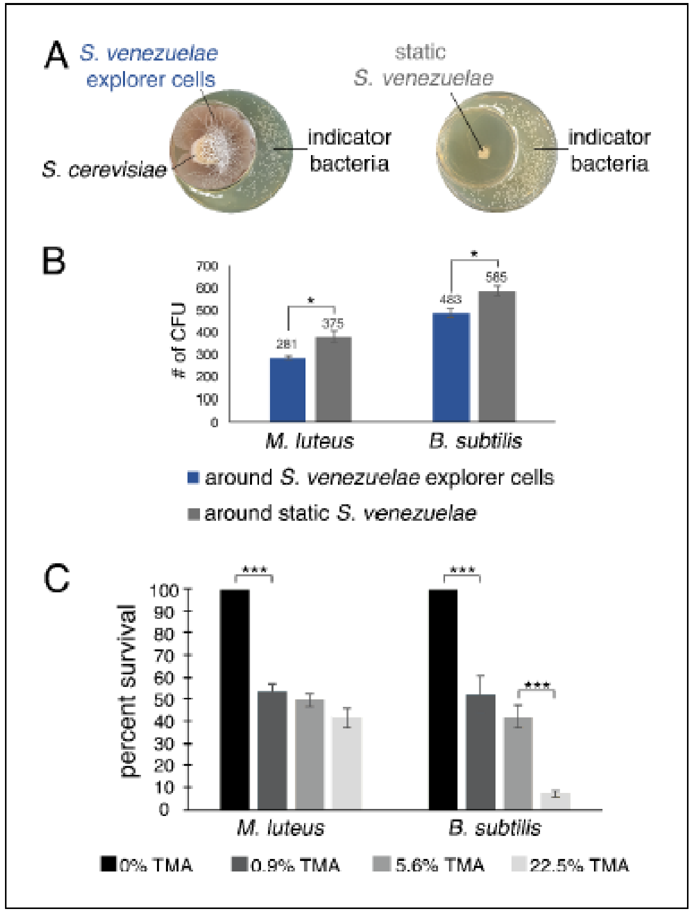


VOC-mediated alkalinity stimulates *Streptomyces* exploration

7. Can TMA inhibit growth of other bacteria?

TMA can modify antibiotic resistance profiles of bacteria:

Can release of TMA inhibit the growth of other bacteria?



TMA adversely affected the growth and survival of other soil bacteria

ANTAGONISTIC

i. Antibiosis: Production of antibiotics or antifungal toxins by fungi and bacteria provide competitive advantage

ii. Physical interactions: bacterial aggregation or organized bacterial biofilms on the surface of fungal hyphae are associated with reduced fungal viability

iii. Environmental modifications: e.g., changes in pH can influence hyphae formation

MUTUALLY BENEFICIAL INTERACTIONS

Different species in mixed biofilm environments may protect each other against attacking immune responses or antimicrobial agents

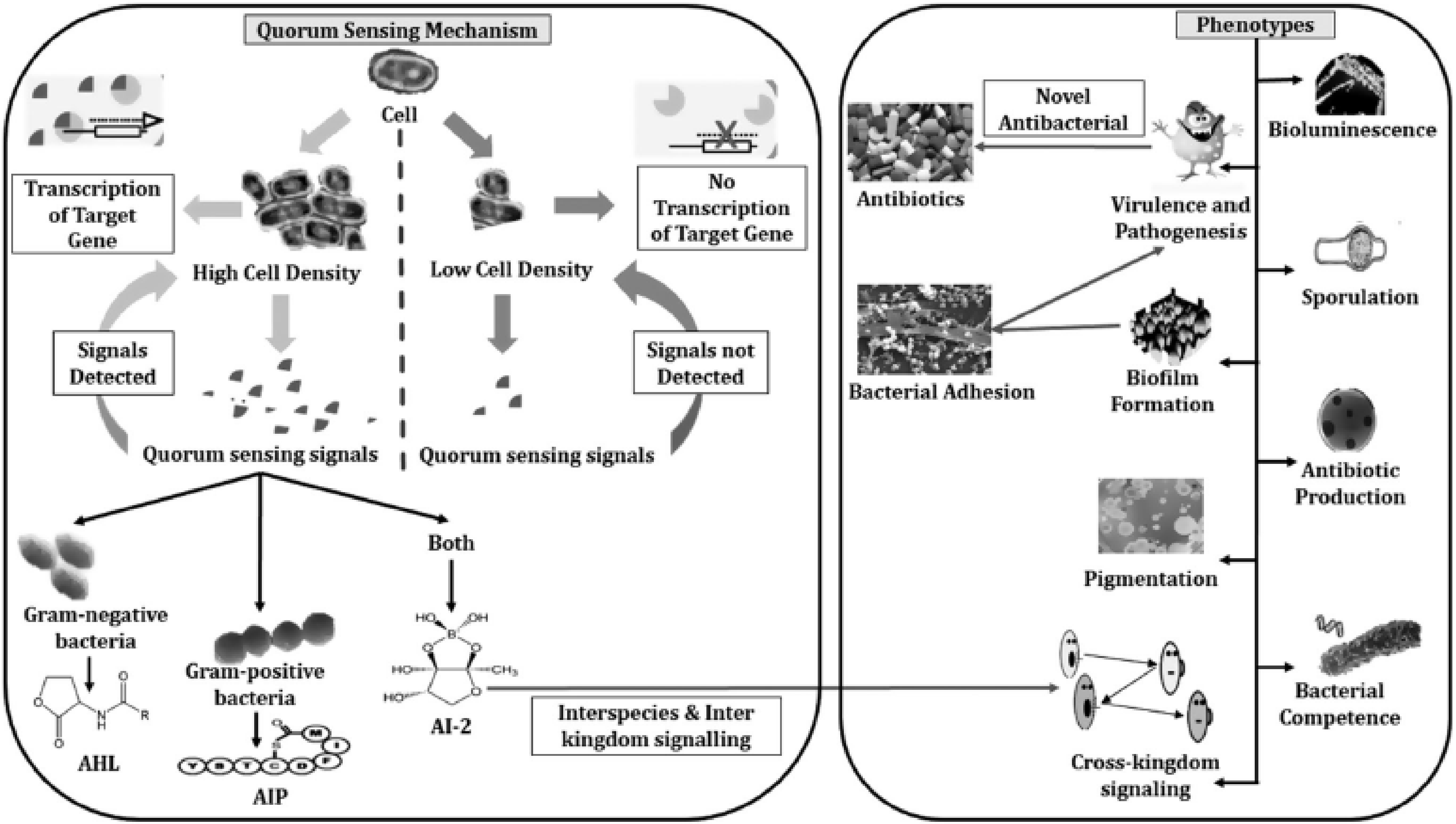


Figure 1. Quorum sensing process and its applications. At high cell density AIs are secreted that are detected by the bacteria to regulate the gene expression. QS controls bioluminescence, expression of virulence genes, biofilm formation, antibiotic production, pigmentation, bacterial competence and cross-domain signalling. AI-2 is involved in cross-domain signalling process.

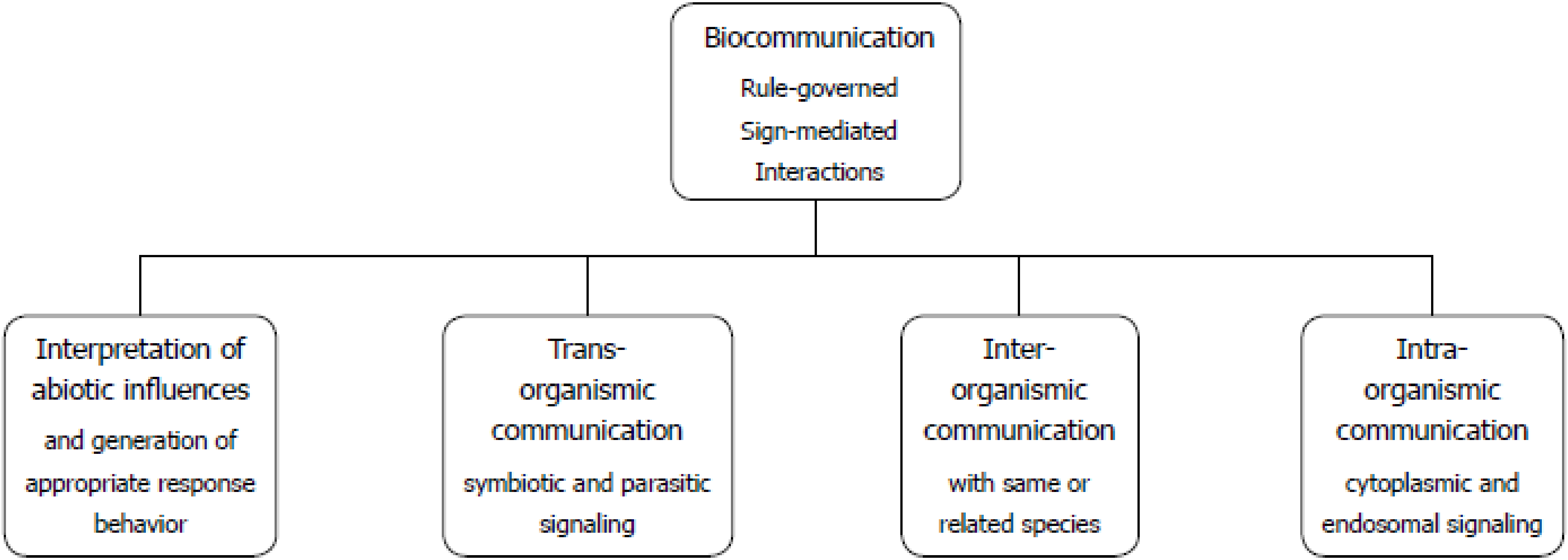


Figure 1 Levels of biocommunicative competences of bacteria, fungi and plants.

The semiochemical vocabulary used by bacteria is diverse:

Acyl homoserine lactones and linear oligopeptides: diverse processes

Cyclized oligopeptides: virulence.

γ-Butyrolactones: used as antibiotics and in sporulation processes.

Furanosyl diester (AI-2): diverse processes and in luminescence

Cis-11-methyl-2-dodecenoic acid (DSF): virulence and pigmentation

4-Hydroxy-2-alkyl quinolines (PQS, HAQs) and palmic acid methyl esters: regulation processes and virulence

Putrescine: swarming motility such as biofilm organization

A-signal: early developmental processes and aggregation

Bipartite Interactions

Bacteria-Fungi

Plant-Bacteria

Fungi-Plant