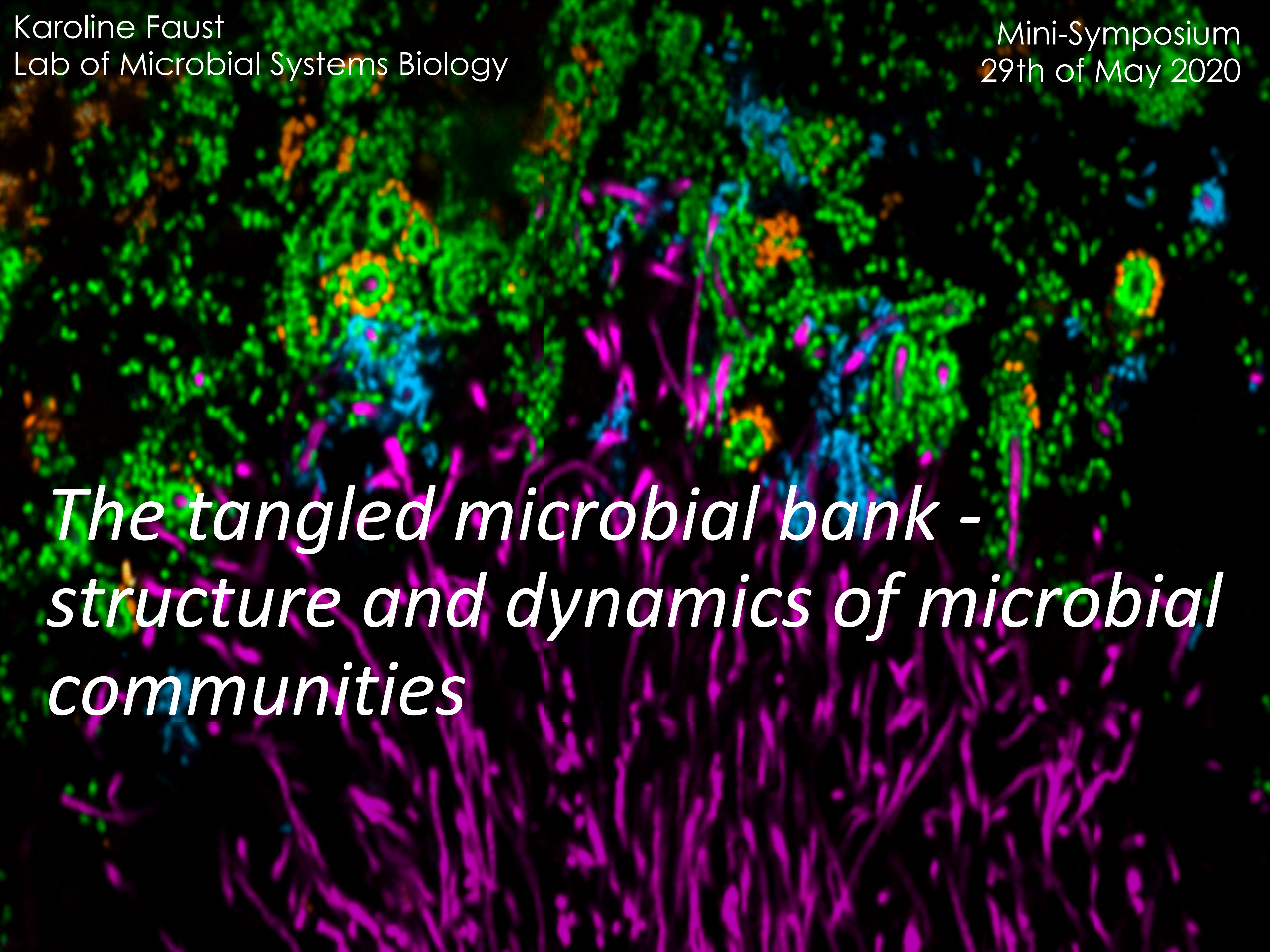
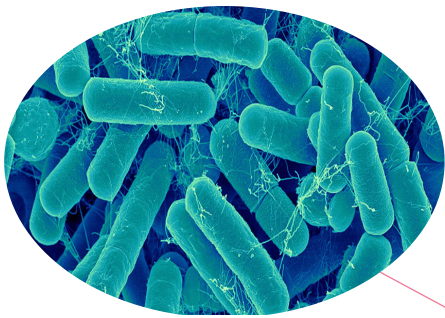


Karoline Faust
Lab of Microbial Systems Biology

Mini-Symposium
29th of May 2020

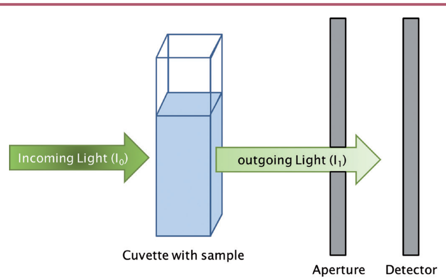
The tangled microbial bank - structure and dynamics of microbial communities

Growing microorganisms



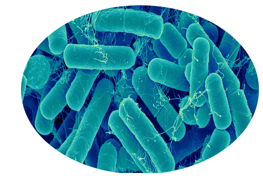
Bioreactor

- Controlled atmosphere
- Controlled temperature
- Well-mixed liquid
- Closed: batch
- In- and outflow: chemostat



Microbial growth: increasing turbidity (optical density = OD) in a photometer

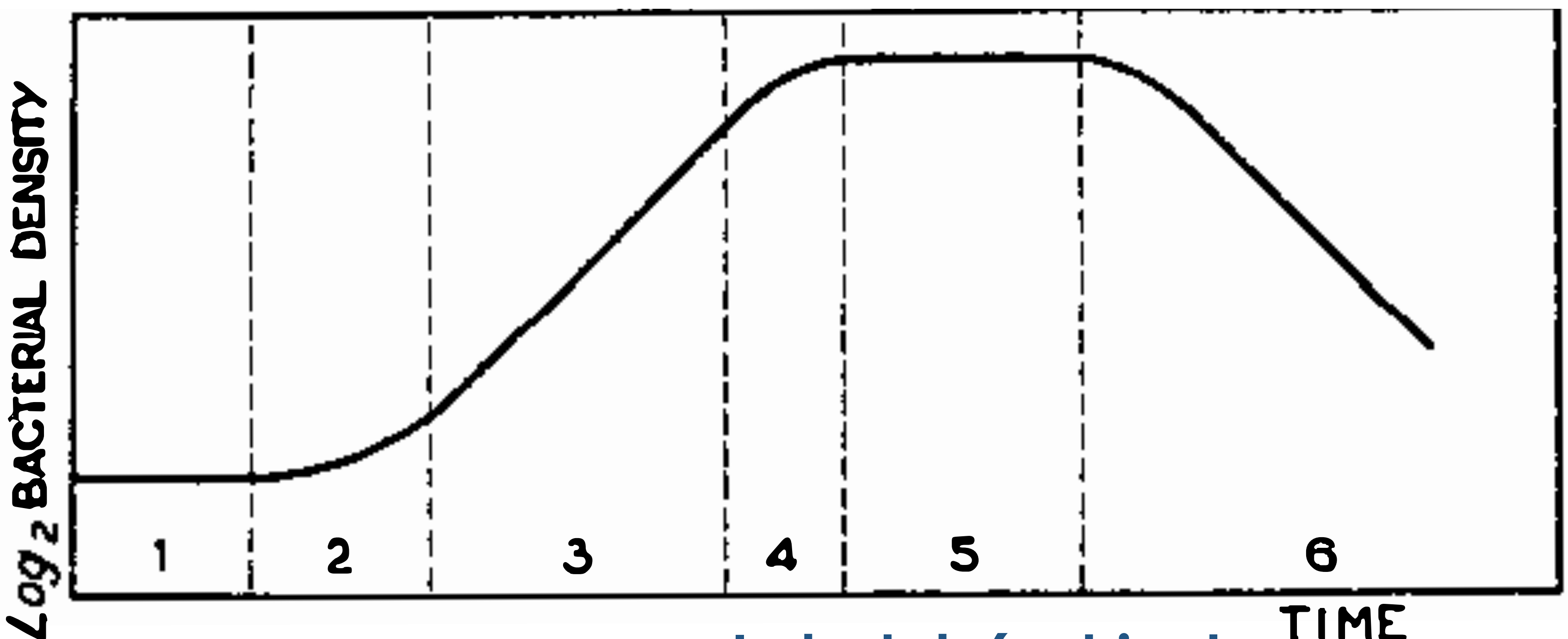
Microbial growth curve



3: **Exponential (Log) phase**

4: Retardation phase
5: **Stationary phase**

6: Phase of decline (**death**)



1: **Lag phase**
2: Acceleration phase

In batch (nutrient is depleted)

Figure taken from Jacques Monod: "The Growth of Bacterial Cultures", Annual Rev. Microbiology 1949, 371-394.

Logistic equation

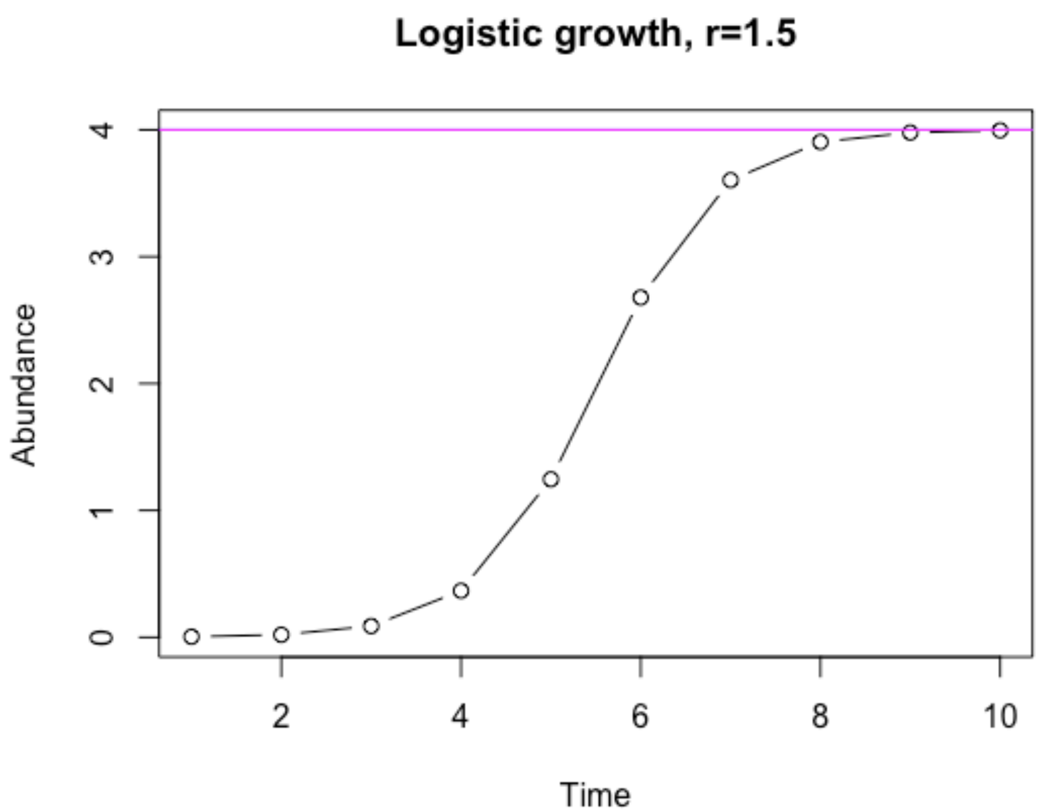
- In batch, bacteria enter stationary phase when they run out of food
- The less food they have, the slower they grow
- Bacterial biomass is constrained (**carrying capacity**)
- Logistic equation describes this behaviour:

x: Biomass
K: Carrying capacity
r: (Intrinsic) growth rate

$$\frac{dx}{dt} = \mathrm{r}\left(\frac{K-x}{K}\right)x \Leftrightarrow \frac{dx}{dt} = \mathrm{r}\left(1-\frac{x}{K}\right)x$$

Logistic growth

S-shaped (sigmoid) curve



Carrying capacity

$$\frac{dx}{dt} = r\left(\frac{K-x}{K}\right)x$$

Monod kinetics

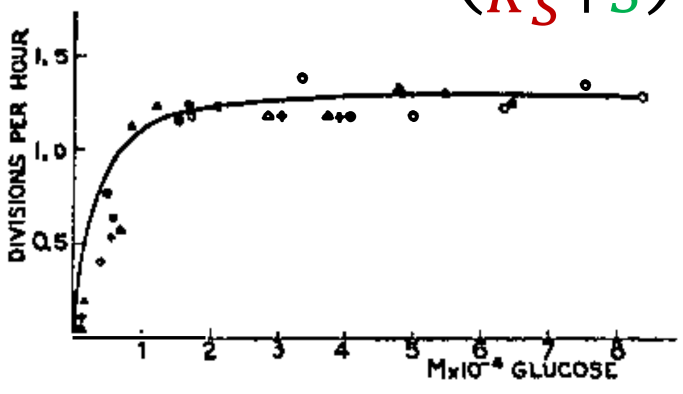
- Carrying capacity depends on available nutrients
- Monod equation describes how growth depends on rate-limiting nutrient
- Monod equation also assumes that with more and more substrate bacteria benefit less and less (saturation)

$$\frac{dx}{dt} = \mu_{max} x \left(\frac{S}{K_S + S}\right)$$

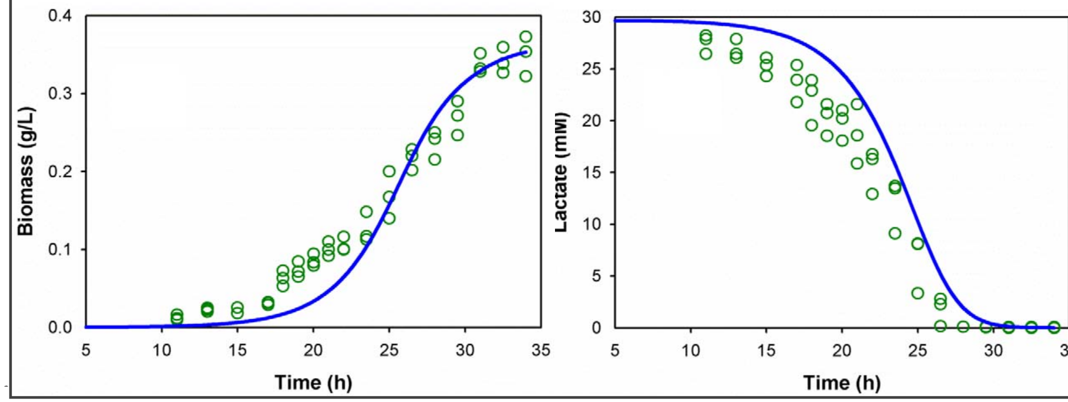
S: Substrate concentration
x: Biomass
K_S: Saturation/Monod constant
μ_{max}: maximum specific growth rate

Monod kinetics - examples

$$\mu = \mu_{max}\left(\frac{S}{K_S + S}\right)$$



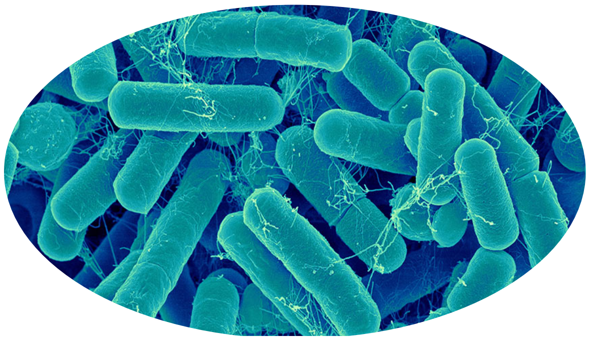
$$\frac{dx}{dt} = \mu_{max}\, x \left(\frac{S}{K_S + S}\right)$$



Example from Monod 1949: **Growth rate change** of *E. coli* with glucose concentration. Increase in growth rate slows down with increasing substrate concentration: **saturation kinetics**

Example from Feng et al. 2012: **Biomass change** in *Shewanella oneidensis* in batch modeled with Monod equation. Decreasing substrate (lactate) slows down biomass increase non-linearly (S changes as a function of x).

More than one microbial species…



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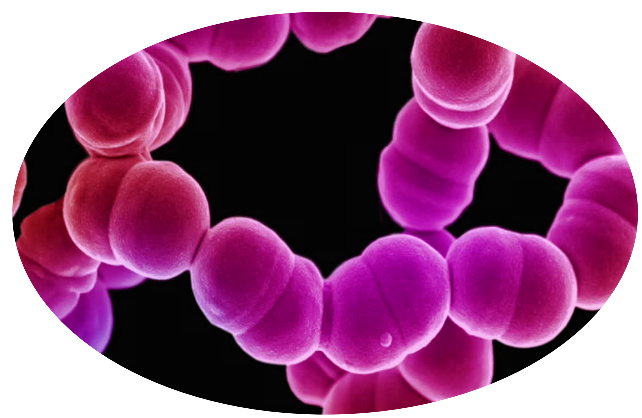


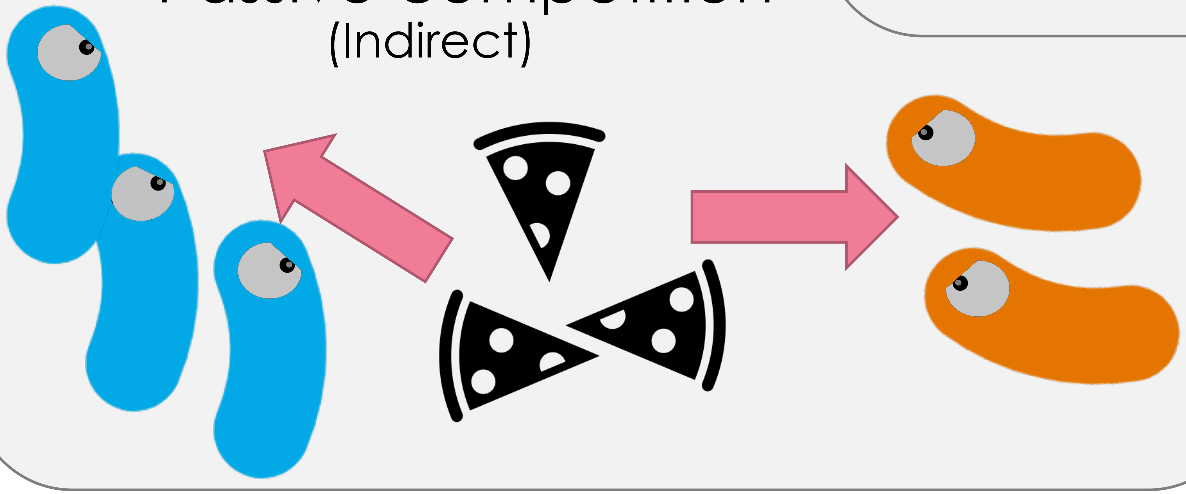
Microorganisms interact: competition

Interference competition

(Direct)

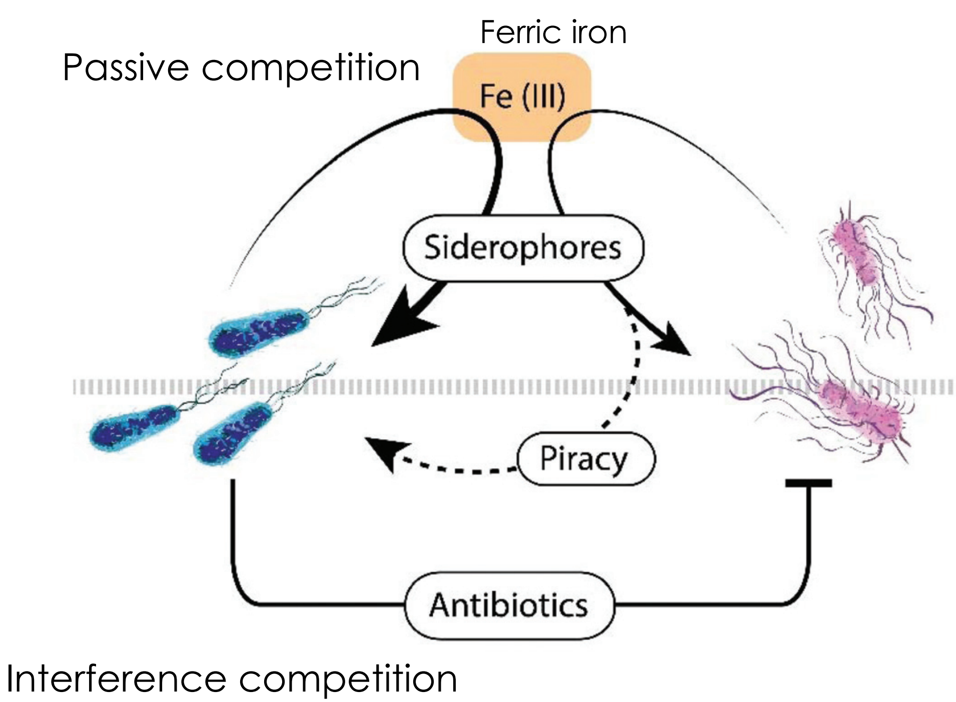
Passive competition

(Indirect)



Microorganisms interact: competition

Example

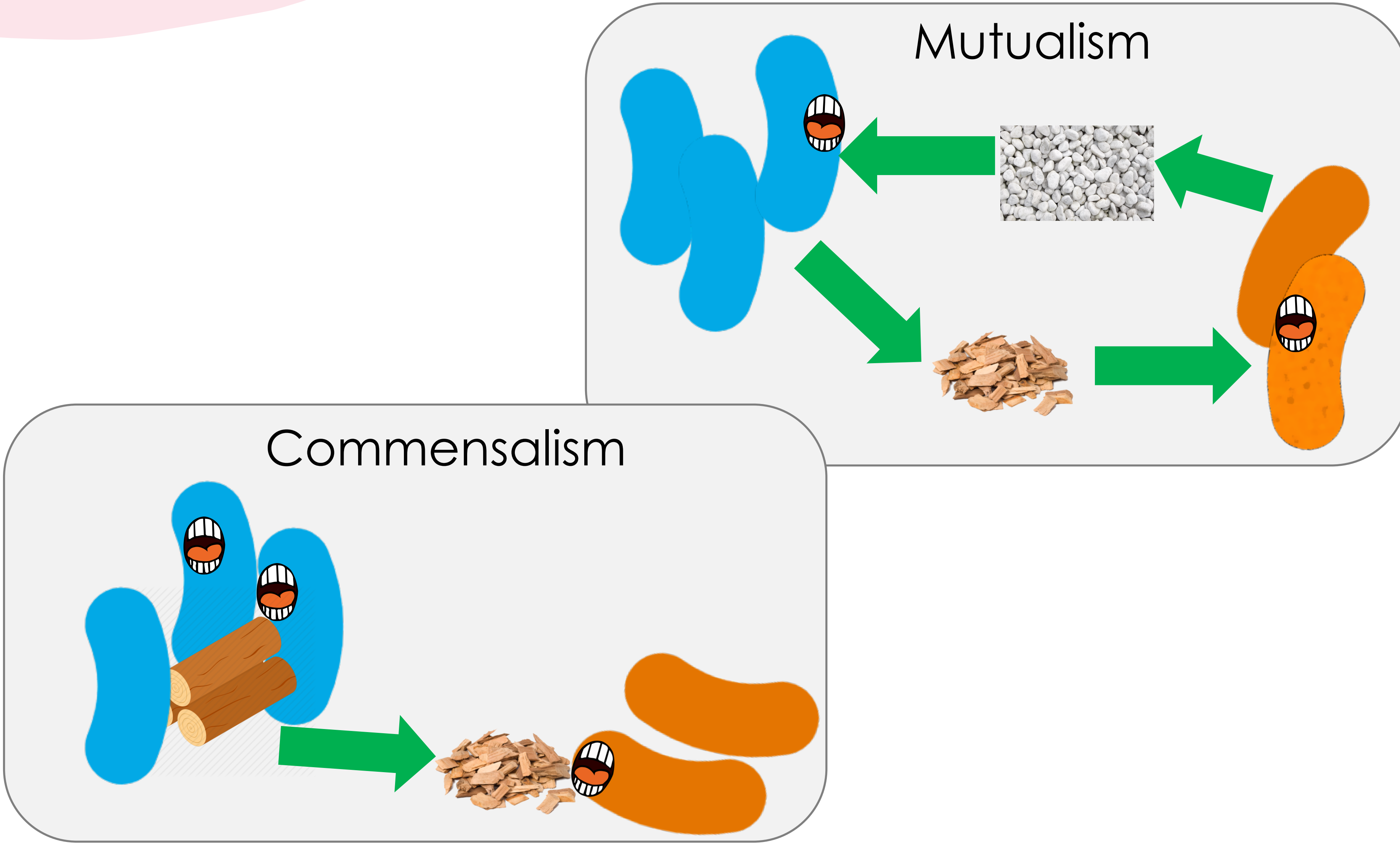


Passive competition: *Pseudomonas aeruginosa* excrete siderophores to transport ferric iron inside the cell, so competitors cannot access it.

Interference competition: *Pseudomonas aeruginosa* produces antibiotics to compete with *Staphylococcus aureus* in the cystic fibrosis lung.

Image taken from Szamosvari et al. Organic & Biomolecular Chemistry 16, 2814 (2018).

Microorganisms interact: cross-feeding



Microorganisms interact: cross-feeding

Example

Sulphate oxidizer, consumes HS^- and produces $SO4^{2-}$

Sulphate reducer, consumes $SO4^{2-}$ and produces HS^-

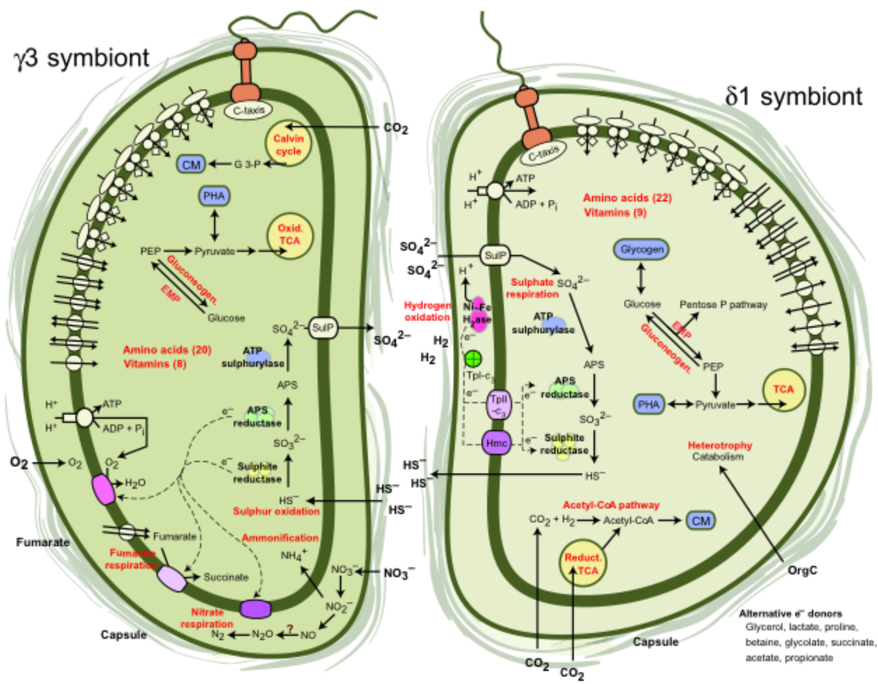
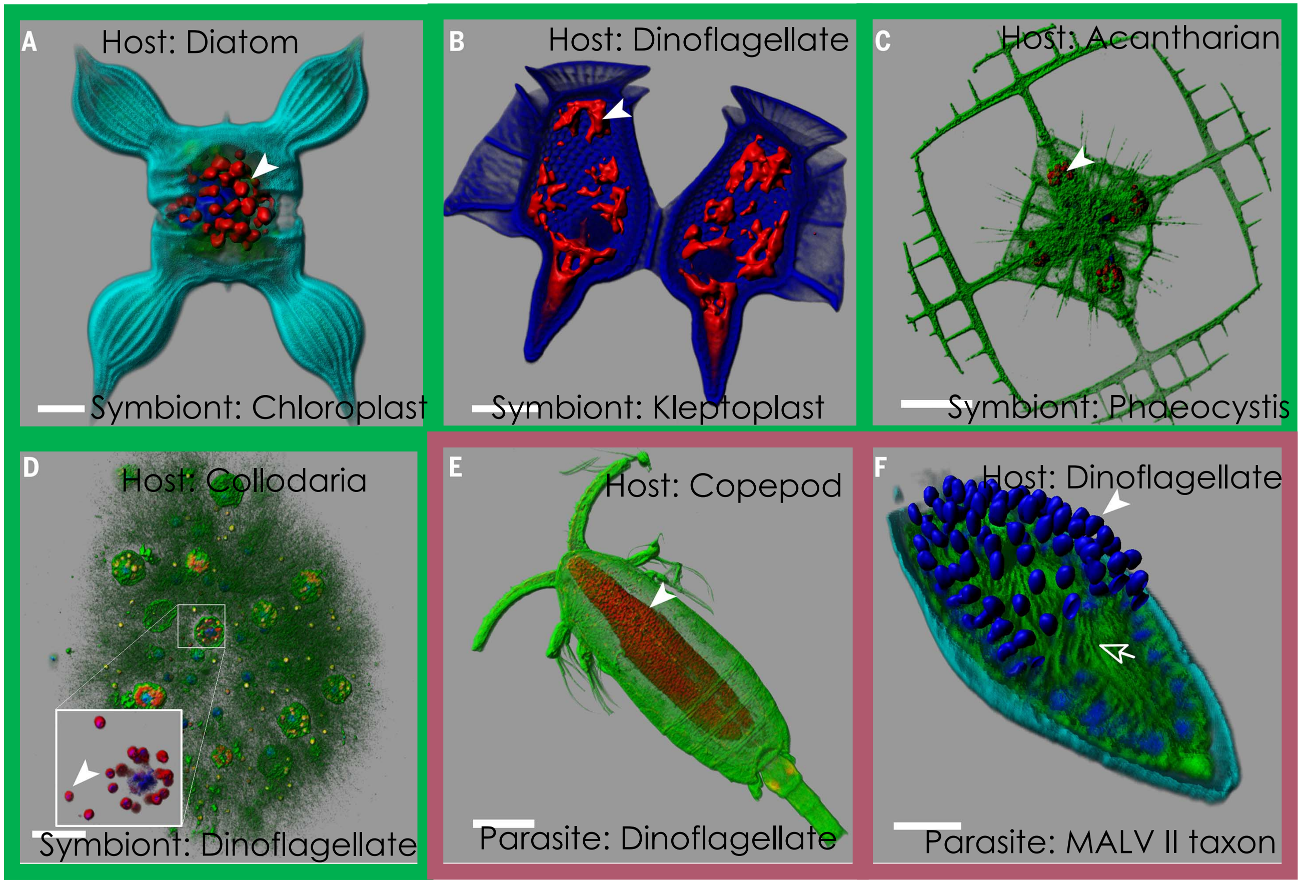


Image source: wikipedia

Marine worm relies entirely on symbionts for feeding (it lacks mouth, gut and anus)

Image taken from Woyke et al. Nature 443, 950-955 (2006).

Microorganisms interact: endosymbiosis



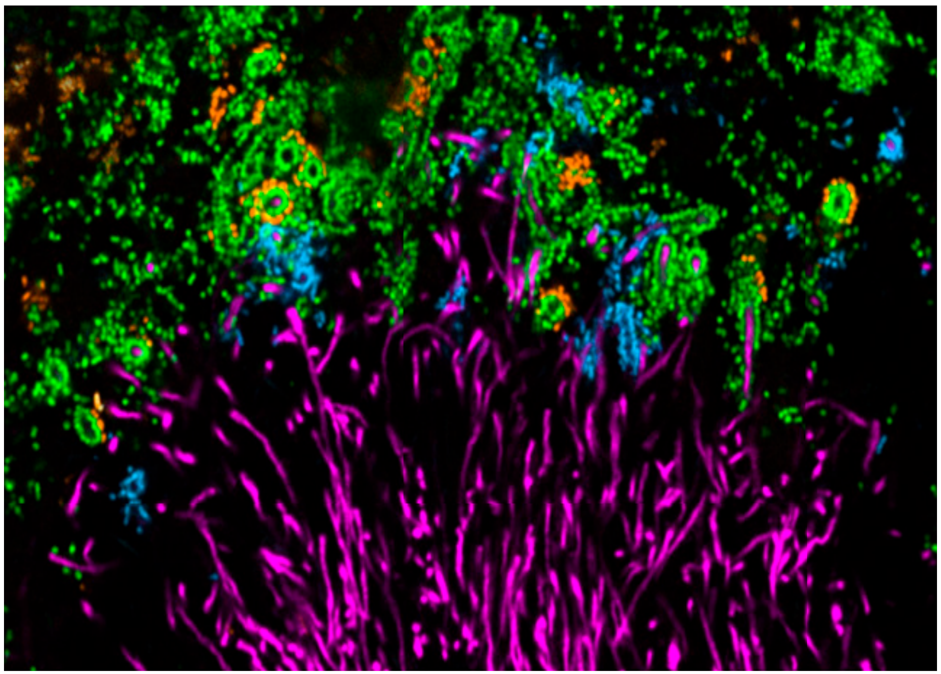
Endosymbiosis

Parasitism

Image taken from de Vargas et al. Science 348, 1261605 (2015).

Microorganisms interact: biofilms

Oxygen and nutrient rich (Saliva)



"Corncob" structures in dental plaque with Corynebacteria filaments at the base and Streptococcus cocci on top

Oxygen and nutrient poor (Tooth)

- *Corynebacterium*
- *Streptococcus*
- *Porphyromonas*
- *Haemophilus/Aggregatibacter*
- *Fusobacterium*
- *Leptotrichia*
- *Capnocytophaga*
- *Neisseriaceae*

Image taken from de Welch et al. PNAS, E791-E800 (2016).

Classifying microbial interactions

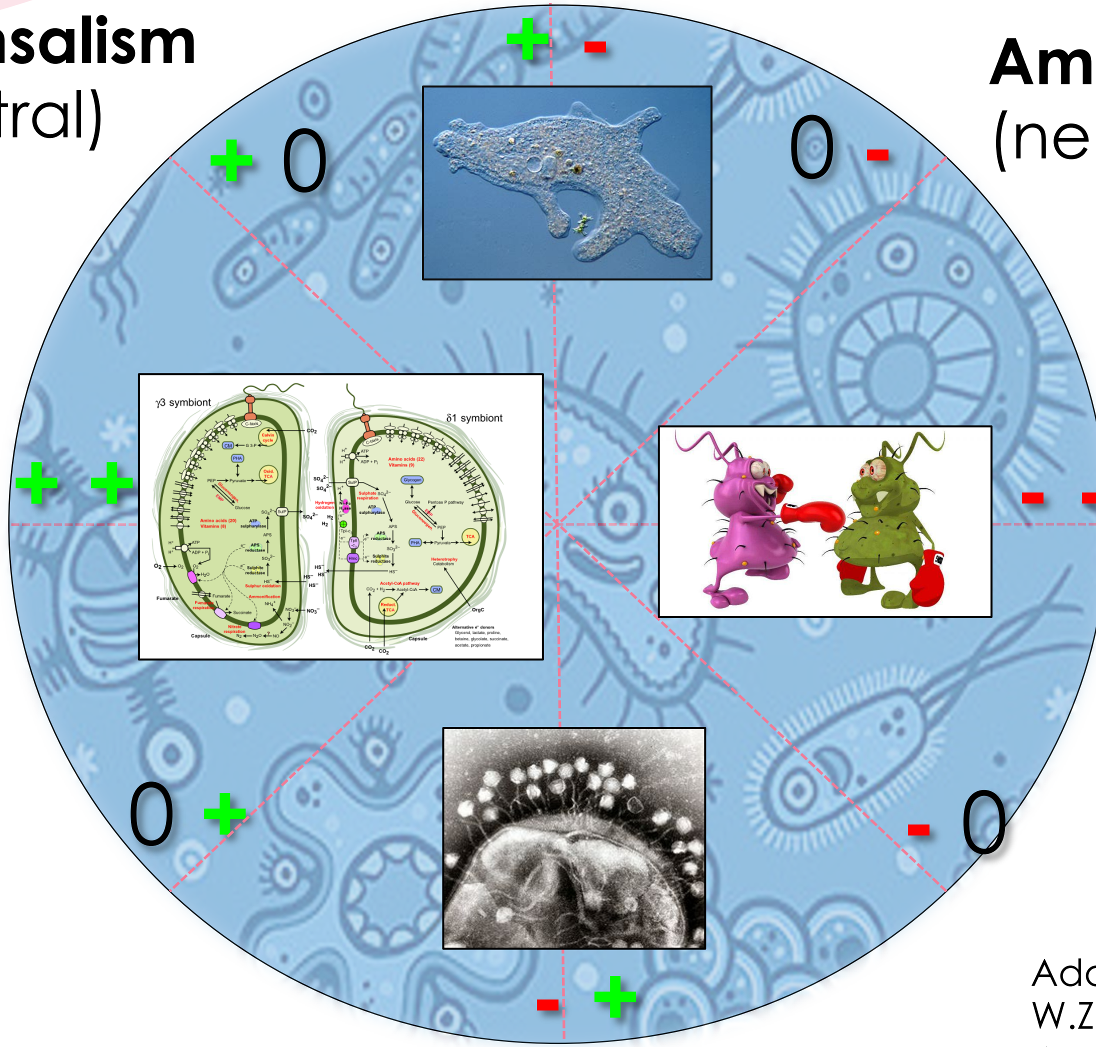
Predator/parasite (win-loss)

Commensalism (win-neutral)

Amensalism (neutral-loss)

Mutualism (win-win)

Competition (loss-loss)



Prey/host (loss-win)

Predation & parasitism = exploitation

Adapted from Lidicker, W.Z. BioScience 29, 475-477 (1979).

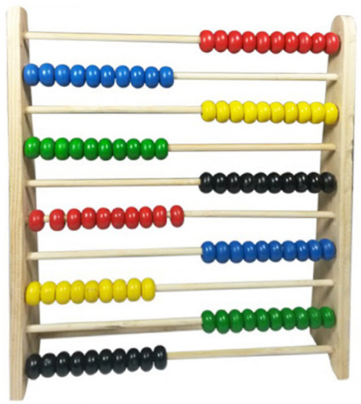
Factors shaping microbial interactions

Specificity	How many potential and actual interaction partners are there?
Space	Particular spatial arrangement or physical contact required?
Environment	Do physical or chemical properties of the environment influence the interaction?
Time	Does interaction depend on a circadian cycle or a particular growth phase?

Modified from: Pacheco & Segrè FEMS Microbiology Letters 366, fnz125 (2019).

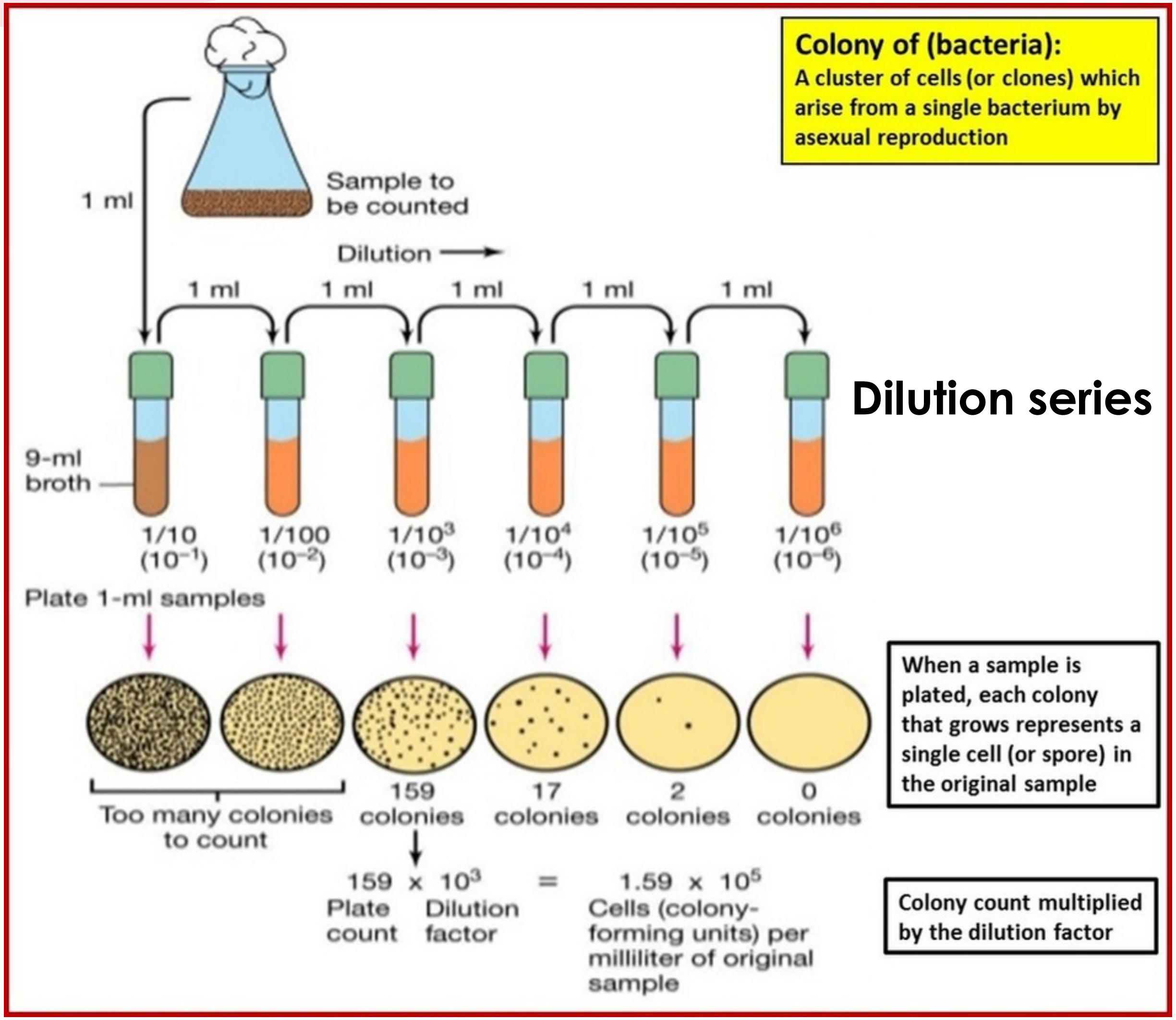
Investigating microbial interactions

- Optical density does not differentiate between different species
- Challenge: We need to count microbial species separately in mixtures



Counting microbes: CFU

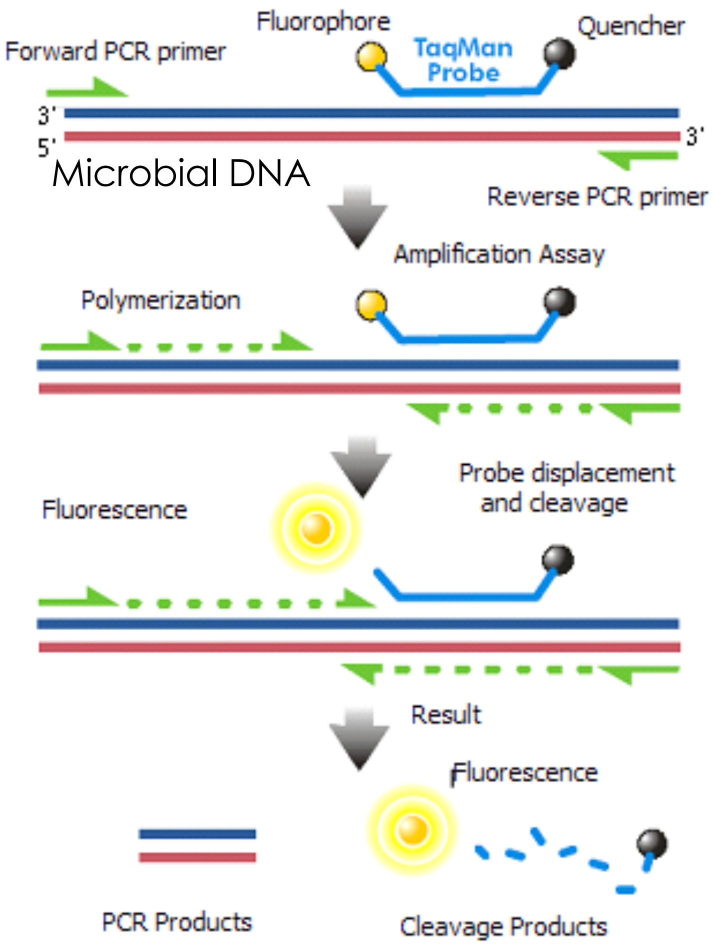
CFU = colony-forming units



- Strains need to be cultivable
- Microbial species can only be counted separately in case morphology differs

Source: http://loretocollegebiology.weebly.com/measuring-bacterial-growth.html#

Counting microbes: quantitative PCR



Source: Wikipedia

- TaqMan: qPCR with species-specific primers and probes
- Quencher suppresses fluorescence
- Primer binds species-specific site
- Taq polymerase extends primer until it reaches probe
- Taqman polymerase cleaves probe, releasing fluorescence

Counting microbes: FISH

FISH = fluorescence in situ hybridization

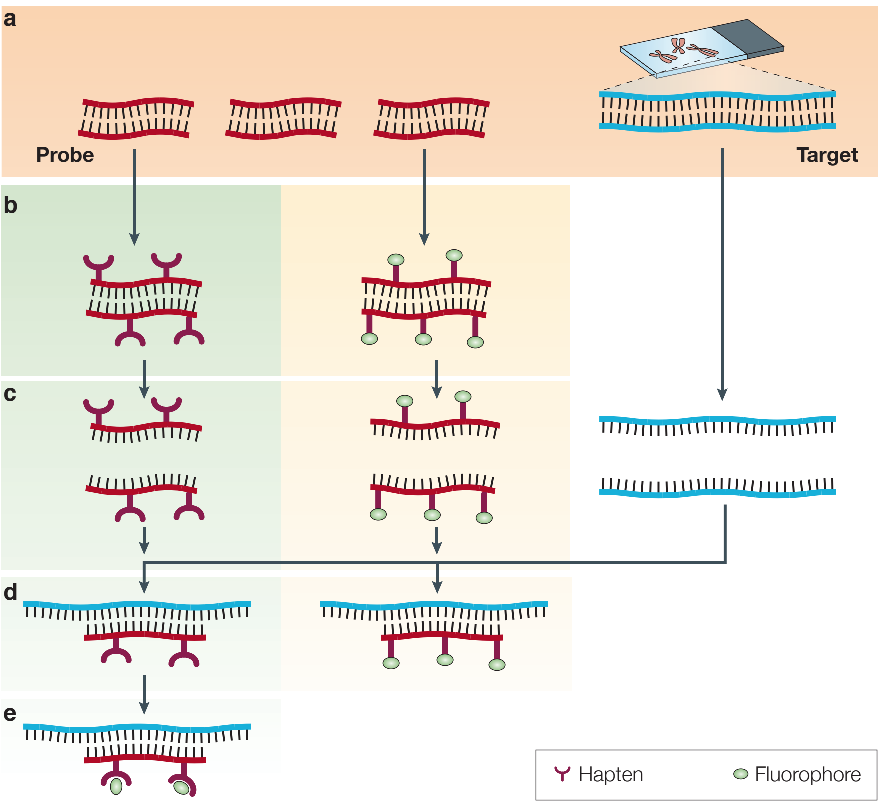
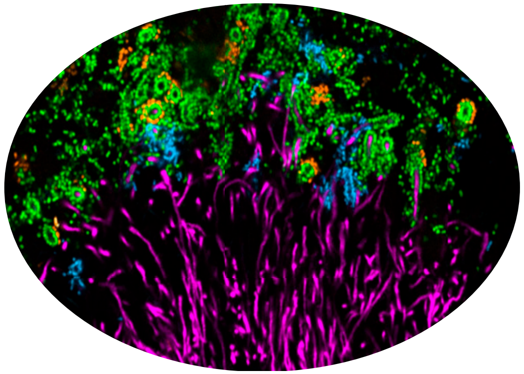


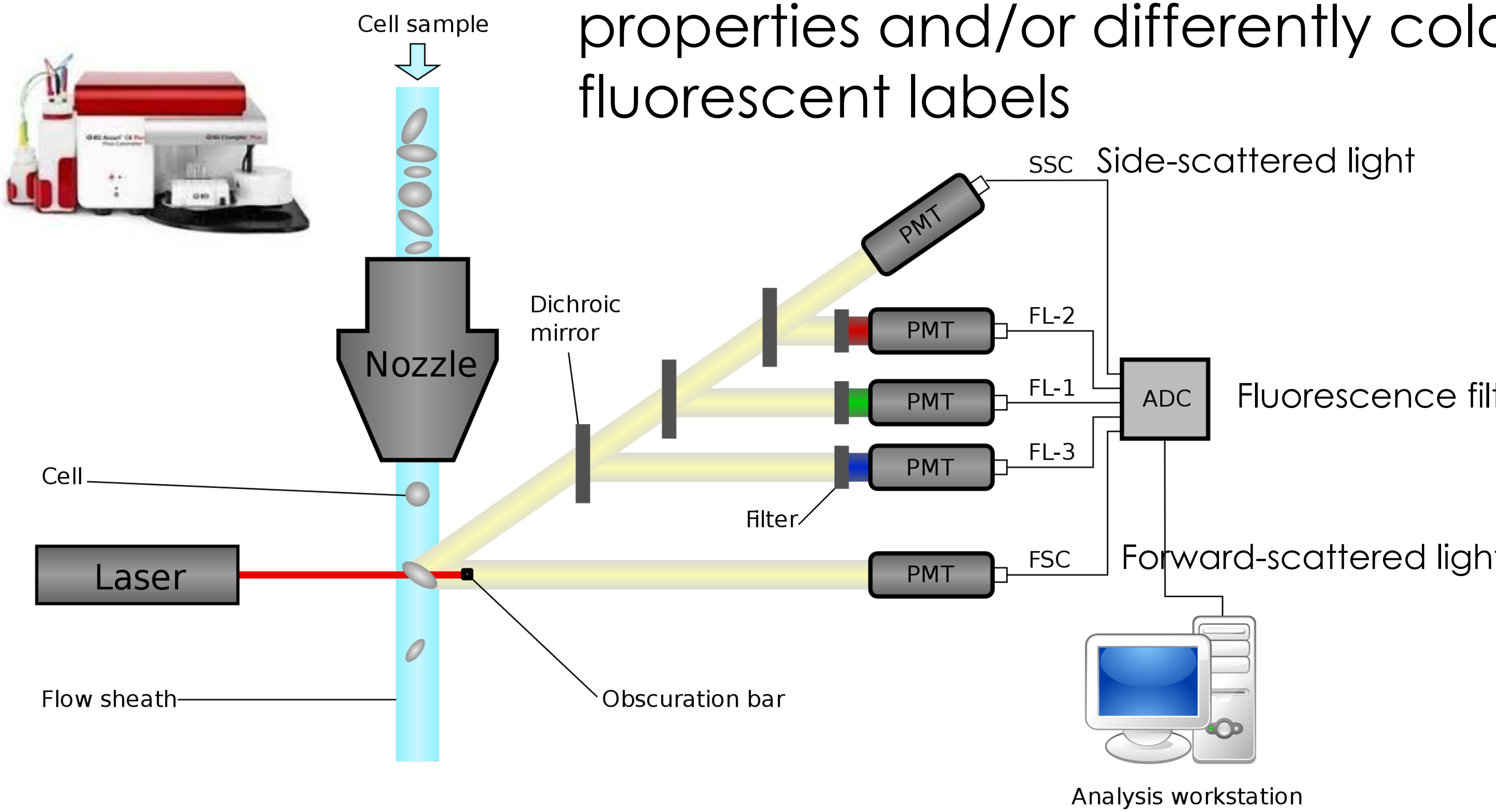
Image taken from Speicher & Carter Nature 6, 782-792 (2005).

- Principle: single-stranded fluorescently labeled probes anneal with denaturated target DNA
- Cells need to be fixated



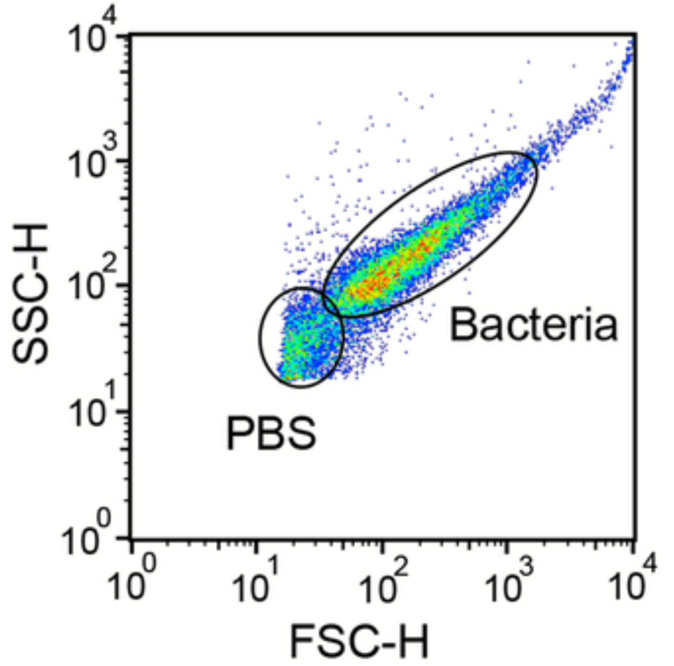
Counting microbes: flow cytometry

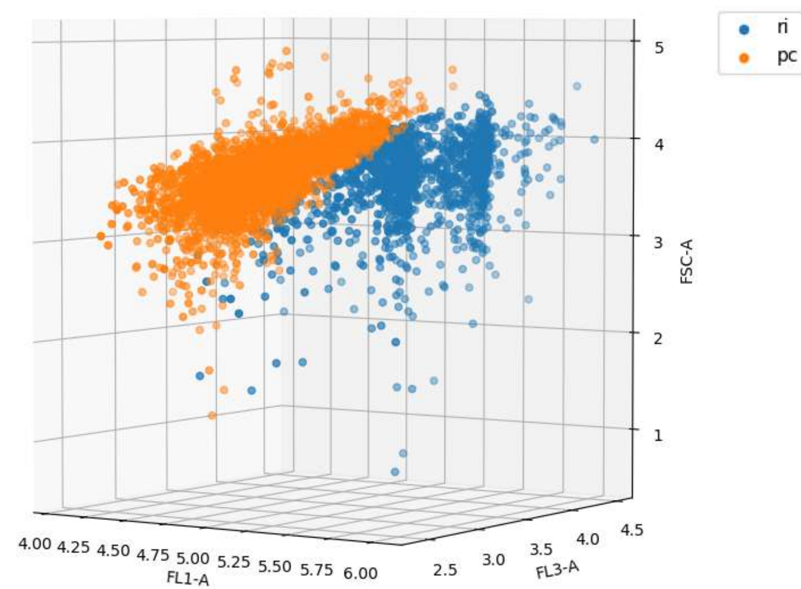
- Counts each cell
- Separates species with different surface properties and/or differently colored fluorescent labels



Source: Wikipedia

Counting microbes: flow cytometry





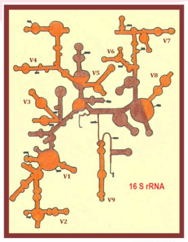
Bacteria: *Staphylococcus aureus*
PBS: Background

Image taken from Gerlach et al. Nature 563, 7733 (2018).

Roseburia intestinalis and *Prevotella copri*

Unpublished data

Counting microbes: sequencing



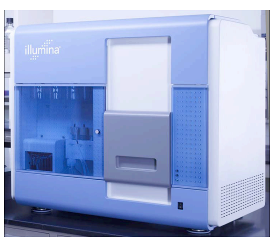
- 16S ribosomal RNA functions as a QR code
- Hypervariable regions: taxonomic classification
- Conserved regions: binding sites for universal primers for DNA amplification

Sampling

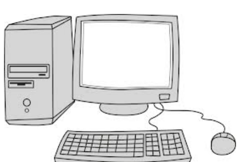
DNA extraction

DNA amplification with universal primers

DNA sequencing



Sequence processing (quality filtering, taxonomic assignment)

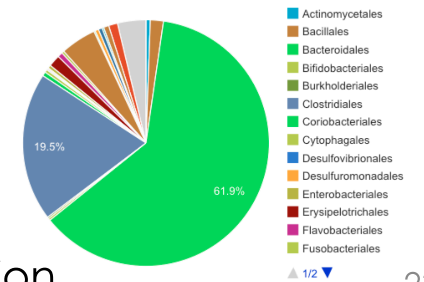


Reference database

16S sequencing workflow

Environment

Microbial composition

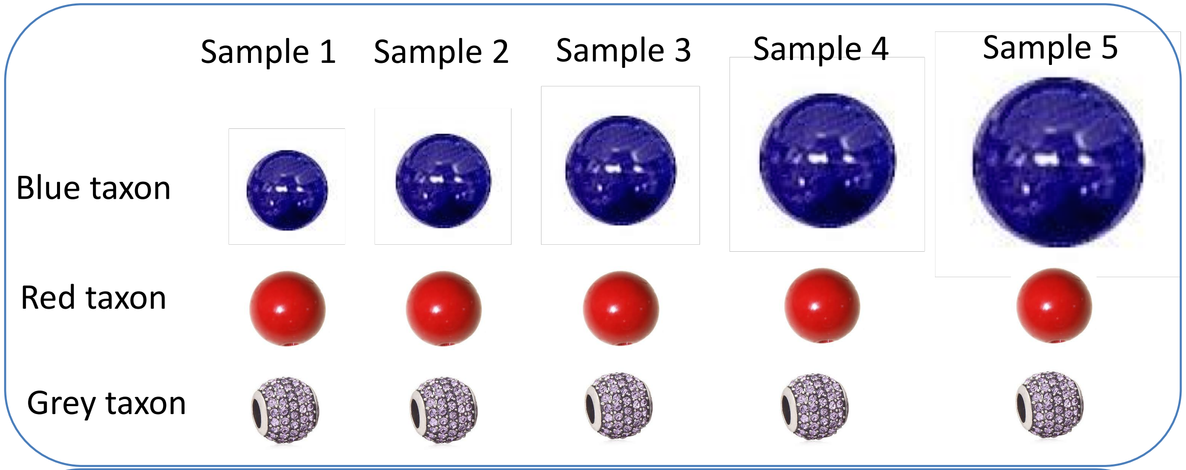


Counting microbes: comparison

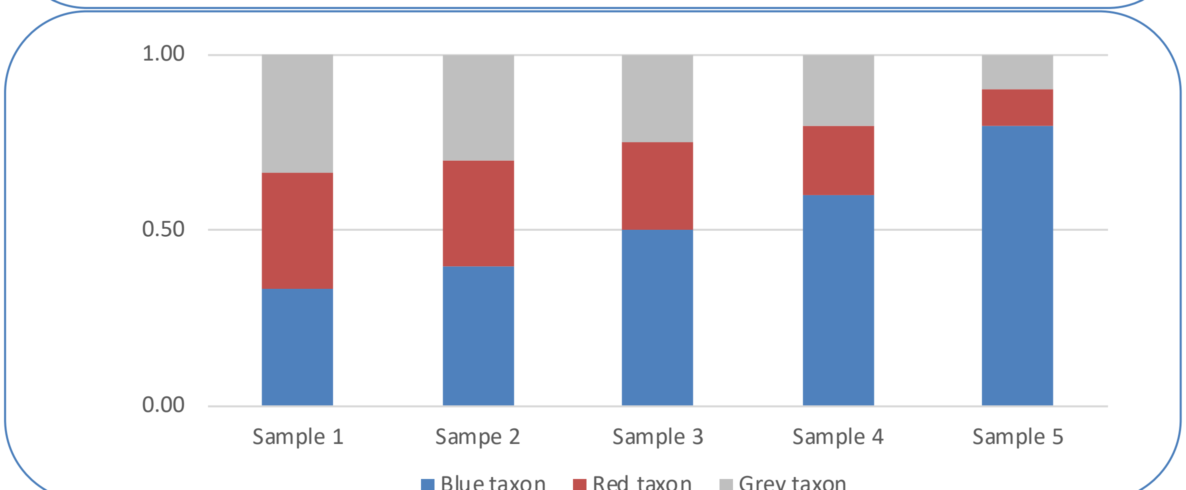
- CFUs, qPCR, FISH and flow cytometry deliver absolute abundances, but do not scale to hundreds of species
- CFU counts living bacteria; other techniques do not differentiate between alive & dead (live/dead staining possible for flow cytometry)
- Sequencing gives only relative abundances, but scales
- Technical variability of sequencing tends to be high

Counting microbes: absolute vs relative

Problem of compositionality



Absolute abundances



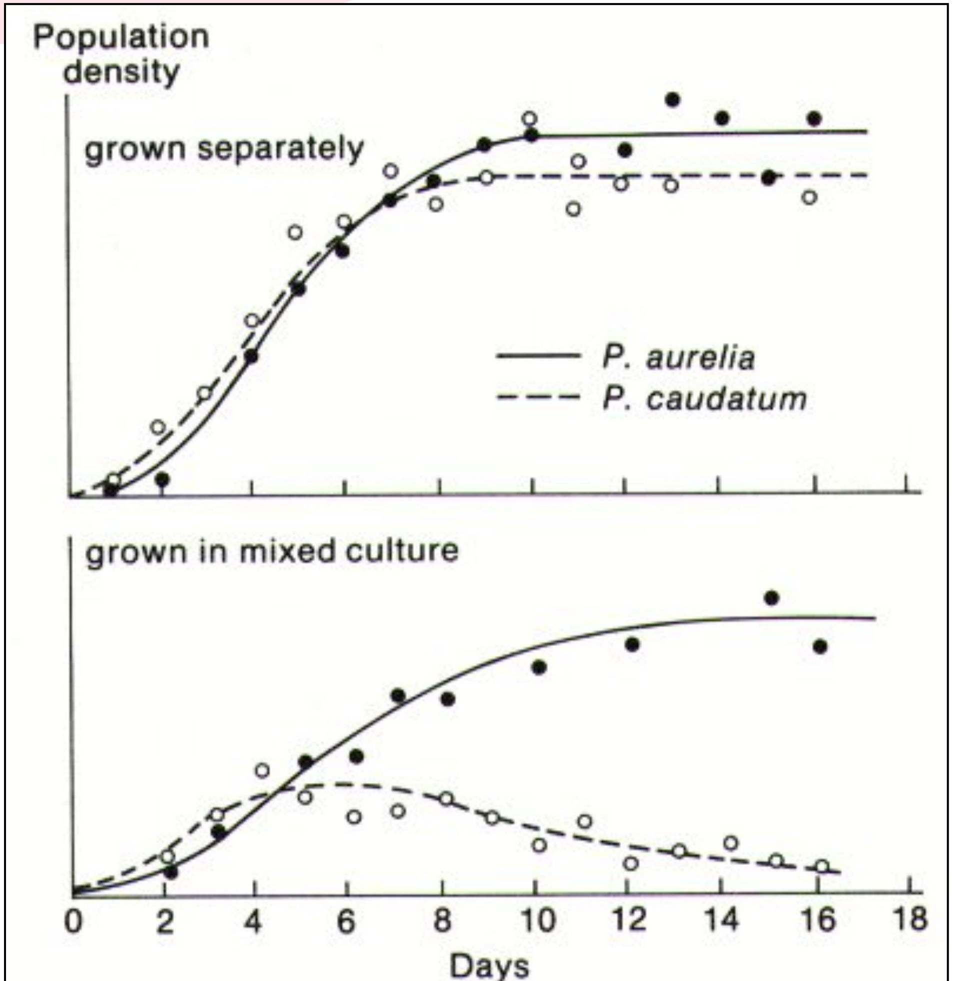
Relative abundances

Investigating microbial interactions

- How do we determine the type and strength of a microbial interaction?

- ➢ Compare growth curves in mono- and co-culture (requires species-specific counts in co-culture and not just OD)
- ➢ Identify interaction mechanism

Compare growth curves



- Compare growth curve in mono- and in co-culture
- Example: *Paramecium aurelia* and *P. caudatum* competition experiment, which led Gause to formulate the competitive exclusion principle

Gause (1934) "The Struggle for Existence", Williams & Wilkins.

Compute interaction strength

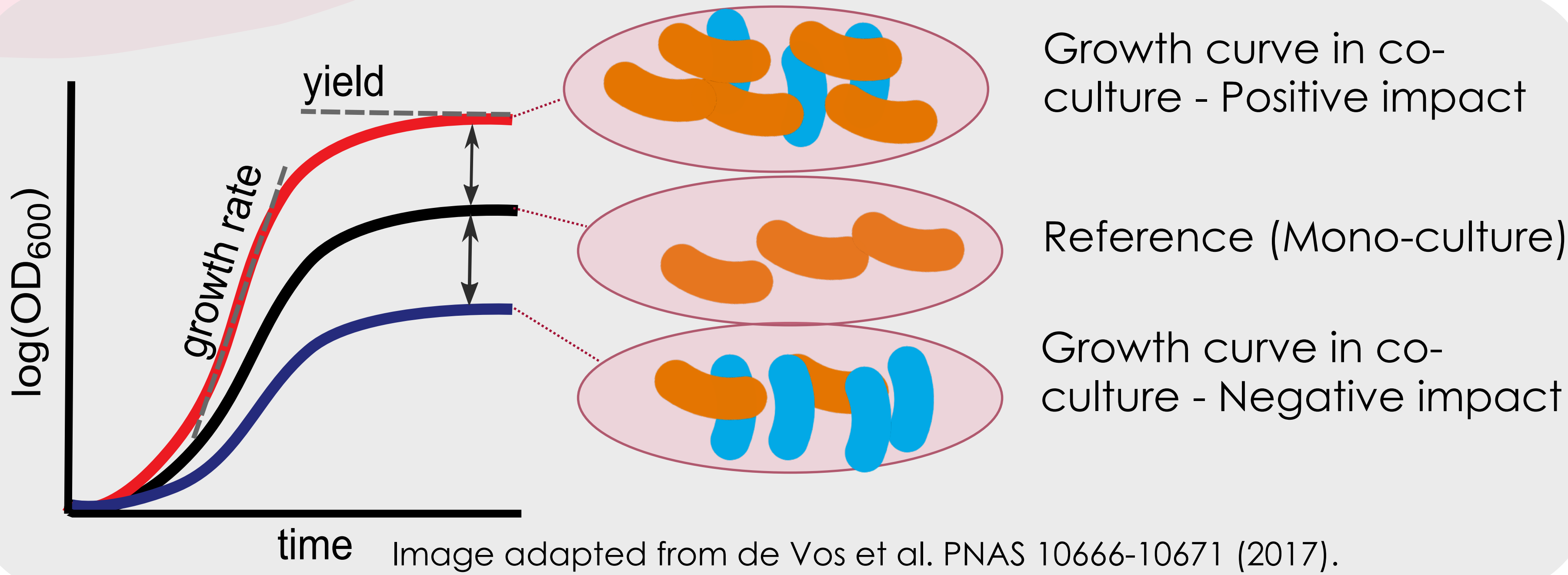


Image adapted from de Vos et al. PNAS 10666-10671 (2017).

Interaction strength computed for both species:

Positive, neutral or negative

$\log\left(\frac{yield\_co}{yield\_mono}\right) >= 0$ $\log\left(\frac{yield\_co}{yield\_mono}\right) < 0$

Interaction type

-,- = competition
-,0 = amensalism
-,+ = exploitation

+,+ = mutualism
+,0 = commensalism
+,- = exploitation

Identify interaction mechanism

Examples

- Genome analysis to identify complementary pathways

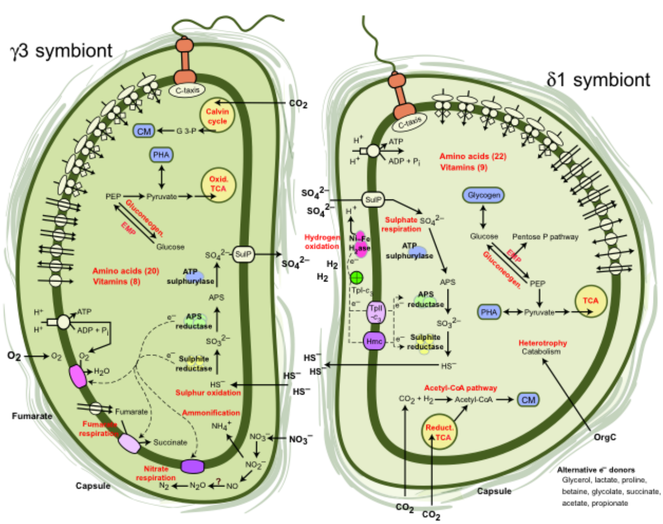


Image taken from Woyke et al. Nature 443, 950-955 (2006).

- Imaging of chemicals involved in interactions

Raman spectroscopy of molecules involved in interference competition

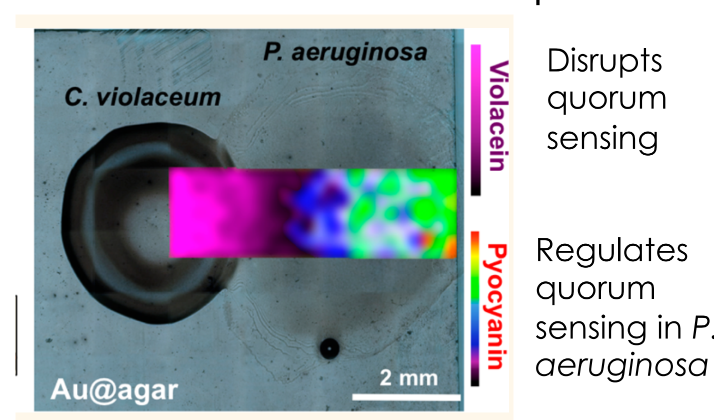
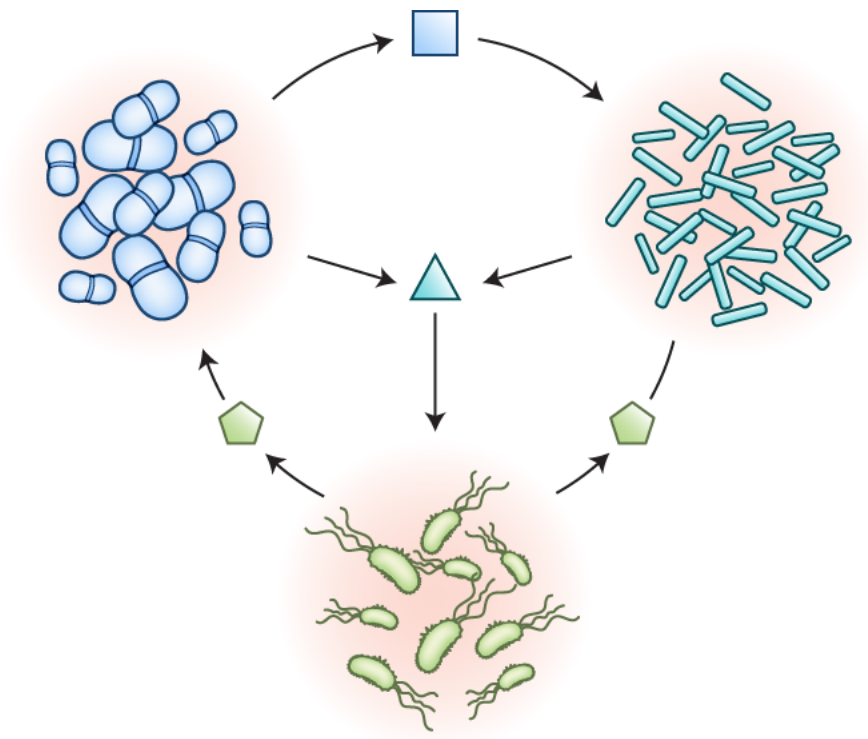


Image taken from Bodelón et al. ACS Nano 11, 4631-4640 (2017).

Can we predict co-culture behavior when interaction mechanisms are known?



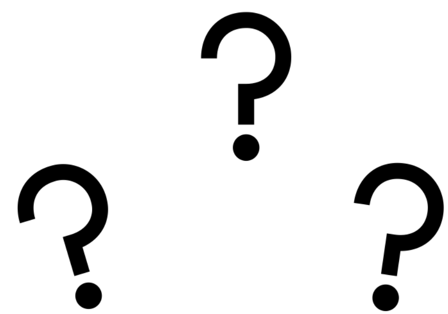
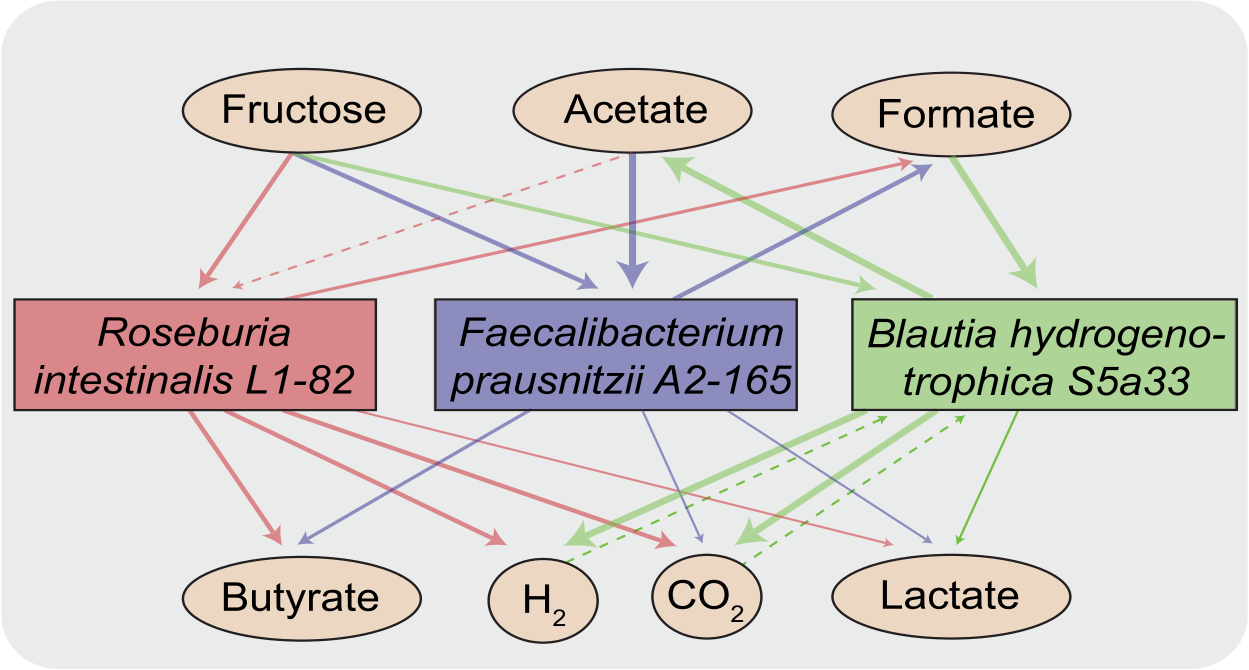


Image taken from Lei Tang, Nature Methods 16, 19 (2019).

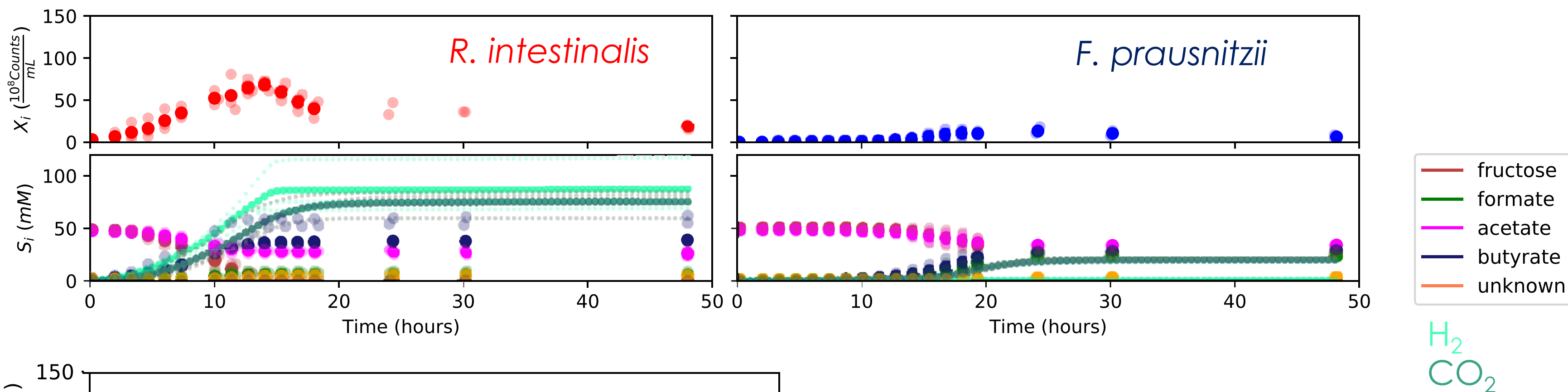
Predicting co-culture behavior from mono-cultures of strains with known interactions

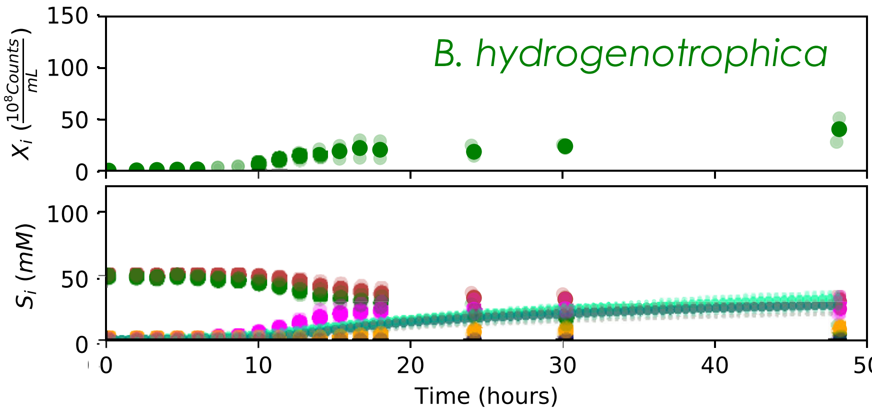
Example: Human gut bacterial community grown in vitro



Predicting co-culture behavior from mono-cultures

- Mono-culture growth curves (qPCR)





➢ Can we predict co-culture growth curves?

Image taken from D'hoe et al. eLife 7, e37090 (2018).

Predicting co-culture behavior from mono-cultures – kinetic model

- Key nutrients consumed and produced are known
- Extend Monod equation to capture more complex behavior

Standard Monod equation: $\frac{dx}{dt} = \mu_{max} x \left(\frac{S}{K_S + S} \right)$

Monod equation for the growth of *R. intestinalis*:

$$\frac{dx_{RI}}{dt} = \mu_{RI} x_{RI} \frac{S_{Fructose}}{K_{RI\_glucose} + S_{Fructose}} \left(1 + \omega_{RI} \frac{S_{Acetate}}{K_{RI\_acetate} + S_{Acetate}} \right) L_{RI}$$

- Acetate boosts growth of *R. intestinalis*, but it can grow without it
- Without fructose, *R. intestinalis* does not grow

L_{RI}: Lag phase function

Predicting co-culture behavior from mono-cultures – model fit

- Mono-culture fit

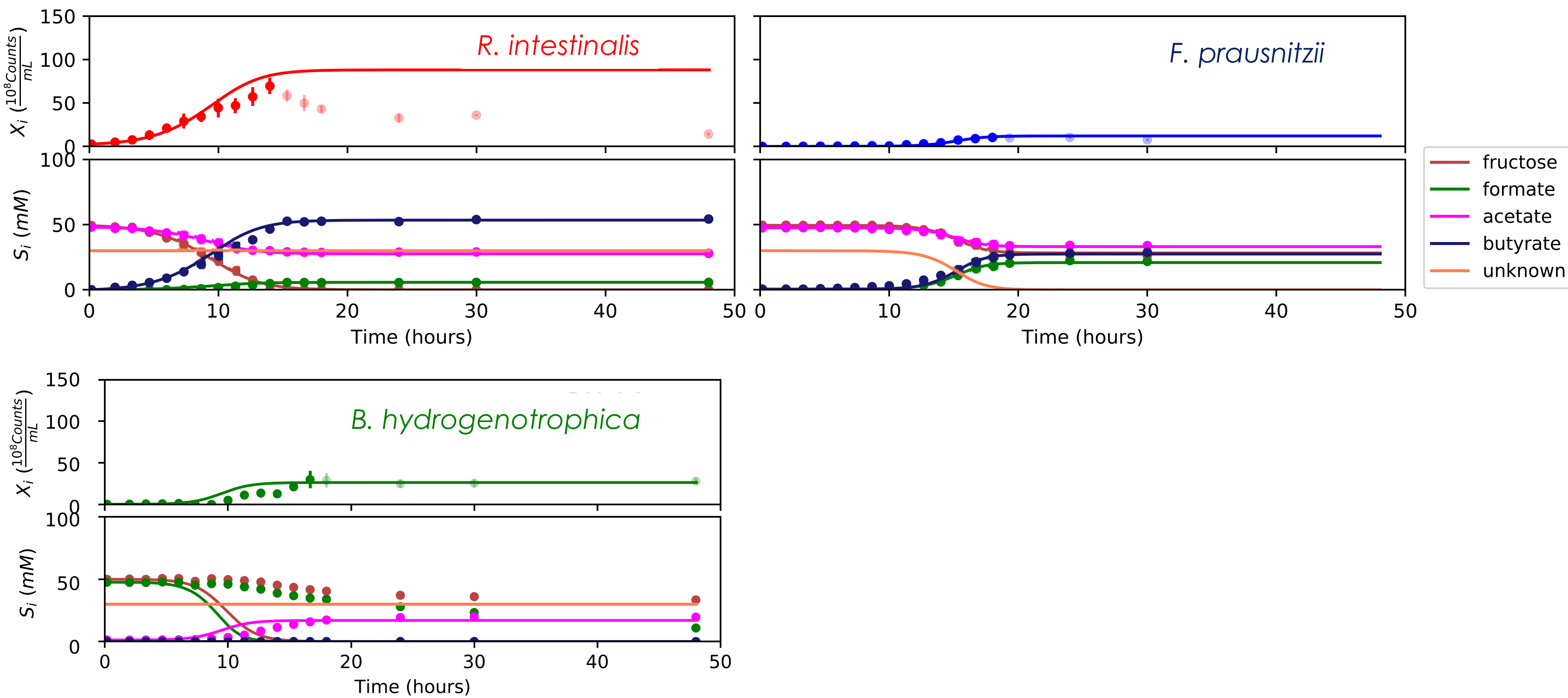
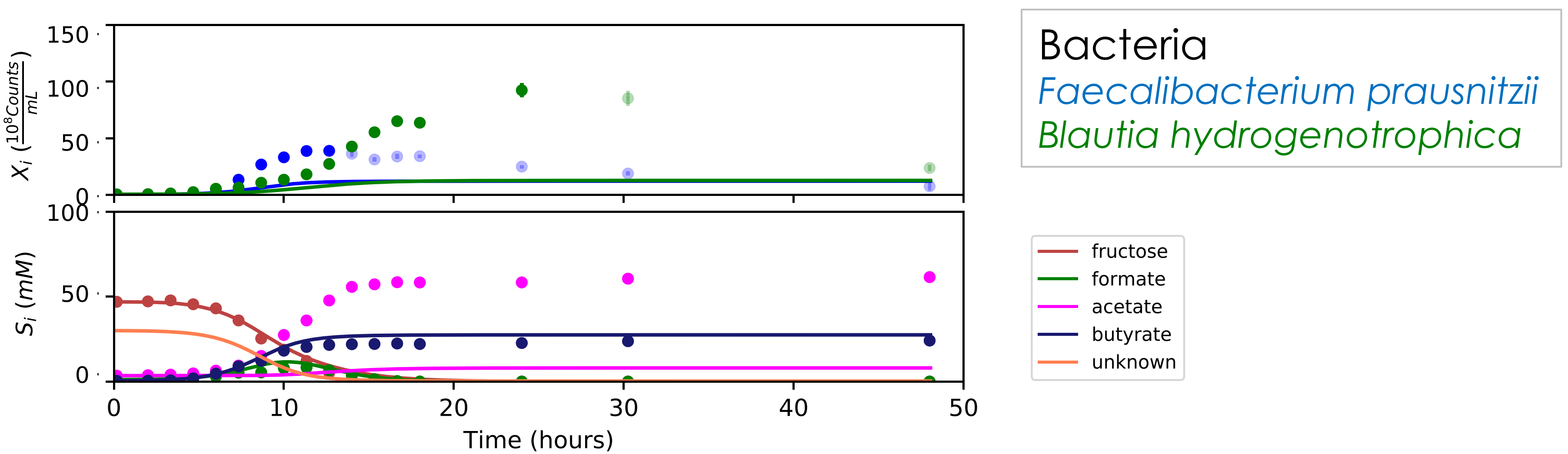


Image taken from D'hoe et al. eLife 7, e37090 (2018).

Predicting co-culture behavior from mono-cultures – model fit

- Co-culture fit



Solid lines: Abundances and concentrations predicted based on mono-culture data
Dots: Observed abundances/concentrations

Both FP and BH reach higher cell numbers than in mono-culture

Image taken from D'hoe et al. eLife 7, e37090 (2018).

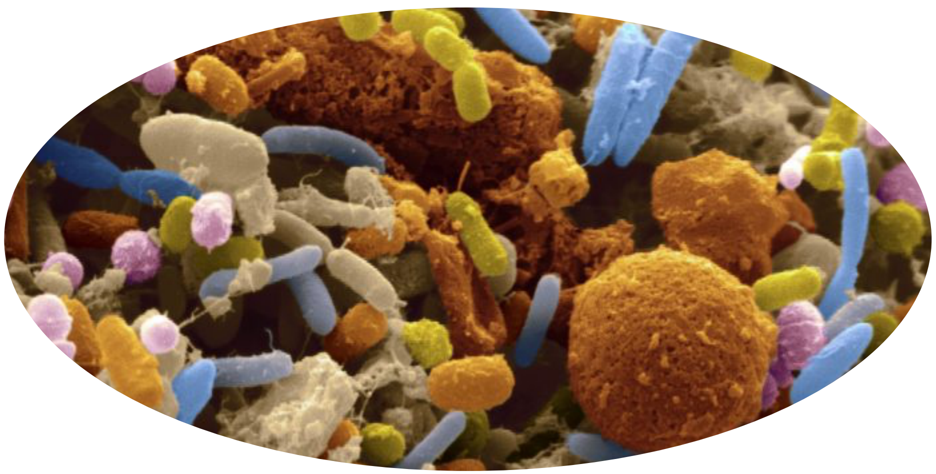
Predicting co-culture behavior from mono-cultures – conclusions

- Metabolic responses to interaction partners can change kinetic parameters
- Kinetic model may therefore be unable to predict co-culture dynamics from mono-cultures
- Metabolic models can deal with metabolic adjustments, but require good knowledge of the metabolism of each community member
- Both kinetic and metabolic models are hard to scale to hundreds of species

Summary part 1: growth, counting & interactions

- Microbial growth curve: lag phase, log phase, stationary phase
- Mathematical models of microbial growth: Logistic equation (ignores substrates) and Monod equation (considers substrates)
- Counting microbes: OD, CFUs, qPCR, FISH, flow cytometry, 16S sequencing
- Ecological interactions: competition, amensalism, mutualism, commensalism, exploitation
- Quantification of interactions: comparison of growth curves in mono- and co-culture
- Bacteria can change metabolism in response to interaction partners such that mono-cultures may not be predictive of co-culture behavior

Can we predict community behavior?



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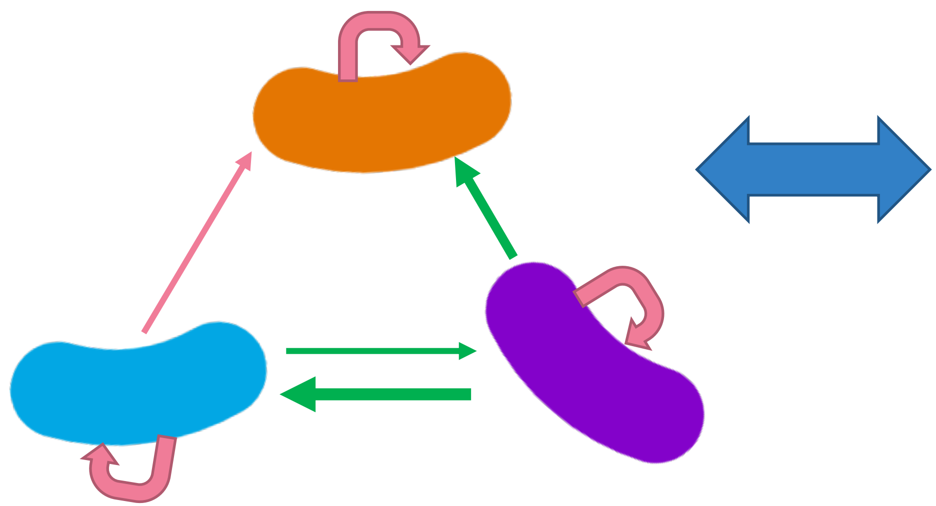
Species interact: community matrix

- The network of interacting species can be represented by the interaction matrix A (also known as community matrix), whose entries represent interaction strengths
- Diagonal: self-interaction strengths

Interaction network



Interaction matrix

	Blue	Purple	Orange
Blue			
Purple			
Orange			

Generalized Lotka Volterra (gLV)

- The change of species abundance x_i over time can be modeled as a function of its growth rate r_i and its interaction strengths a_{ij} with other species *j* and itself

$$\frac{dx_i}{dt} = x_i \left(r_i + \sum_{j=1}^{N} \alpha_{ij} x_j \right)$$

x_i = abundance of species *i*
a_{ij} = interaction strength between species *i* and *j*
r_i = growth rate of species *i*
N = species number

Link between gLV and logistic equation

$$\boxed{\frac{dx_i}{dt} = \left(r_i + \sum_{j=1}^{N} \alpha_{ij} x_j\right) x_i}$$

Generalized Lotka-Volterra

Set inter-species interactions to zero

$$\frac{dx_i}{dt} = (\mathrm{r}_i + \alpha_{ii} x_i)\ x_i$$

Re-arrange growth rate term

$$\frac{dx_i}{dt} = \left(\mathrm{r}_i + \frac{r_i}{r_i} \alpha_{ii} x_i\right) x_i$$

Scaled self-interaction strength is negative

$$\frac{dx_i}{dt} = r_i \left(1 - \frac{\alpha_{ii}}{r_i} x_i\right) x_i$$

Redefine as carrying capacity

$$\boxed{\frac{dx_i}{dt} = \mathrm{r}_i \left(1 - \frac{x_i}{K_i}\right) x_i}$$

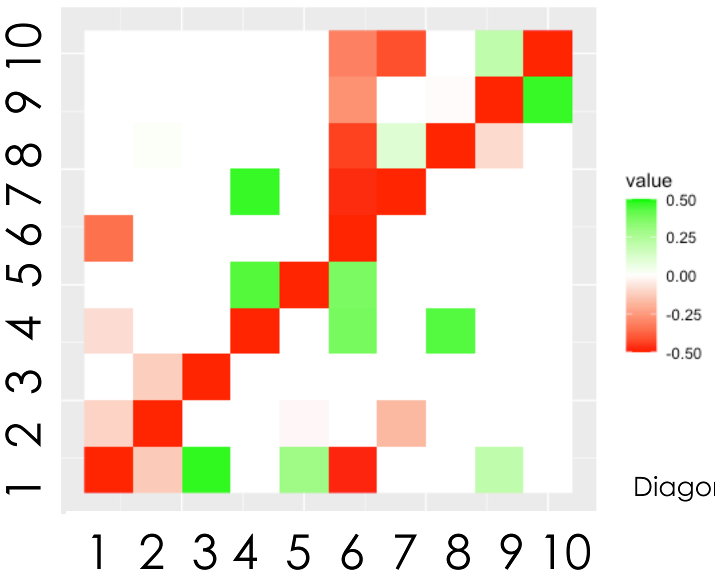
Re-arrange logistic equation

$$\boxed{\frac{dx_i}{dt} = \mathrm{r}_i \left(\frac{K_i - x_i}{K_i}\right) x_i}$$

Self-interaction strength on the diagonal of the interaction matrix = carrying capacity

Simulation with gLV

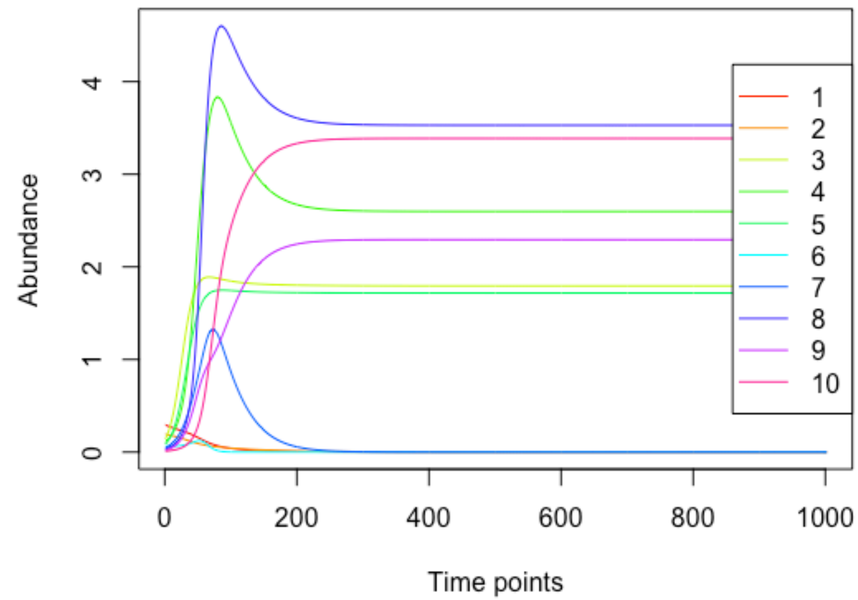
Community matrix

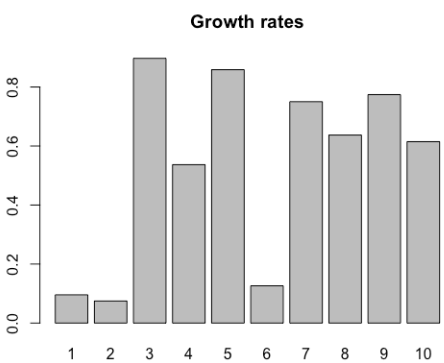


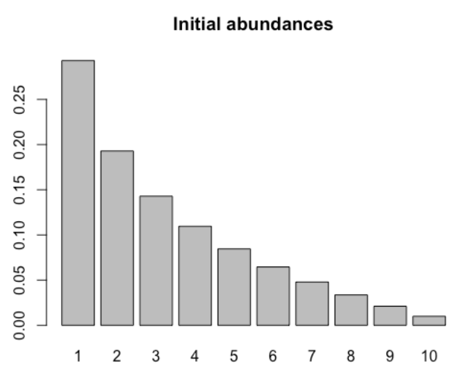
Diagonal: -0.5

Transient

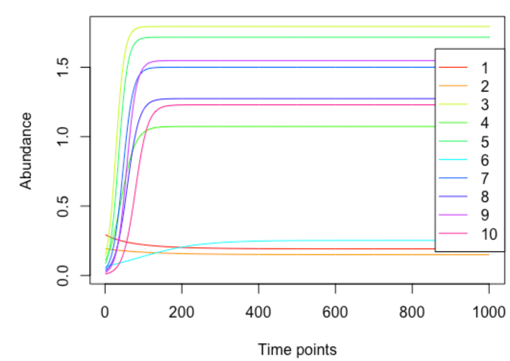
Steady state



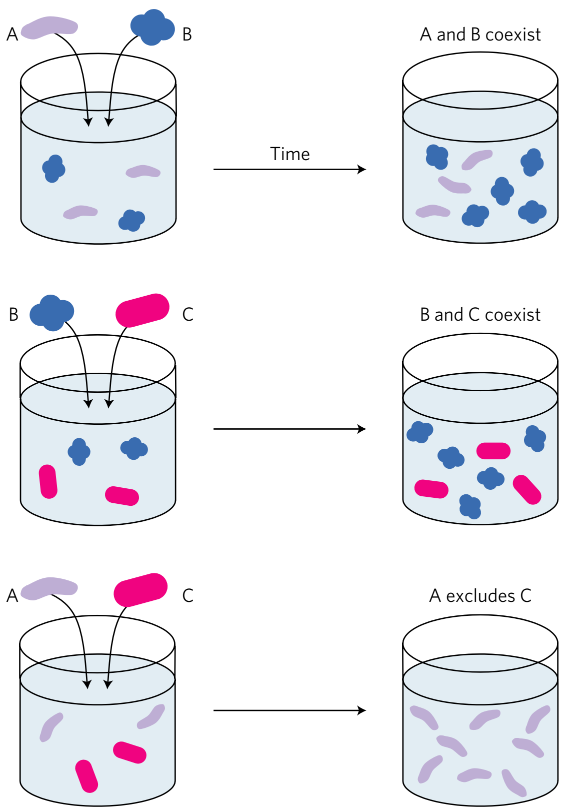




No inter-species interactions:



Can we predict community behavior from pairwise interactions?



gLV assumes that community behavior can be predicted from pairwise interactions

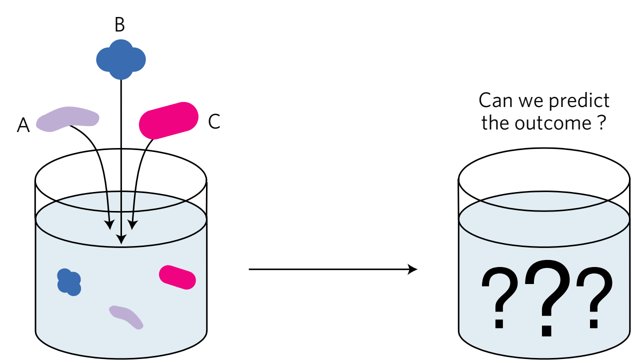
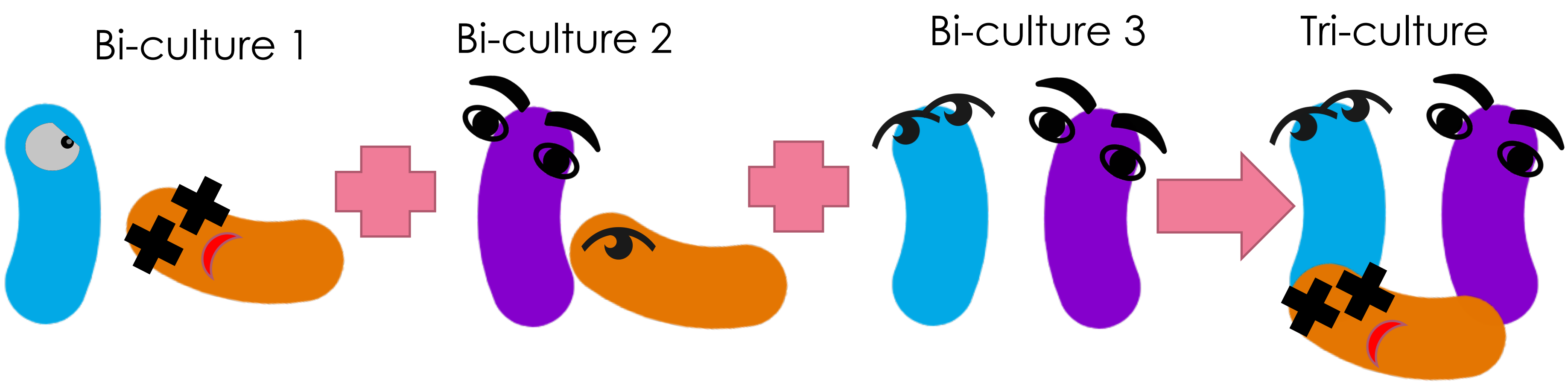


Image taken from Friedman et al. Nature ecology & evolution 1, 0109 (2017).

Can we predict community behavior from pairwise interactions?

- Hypothetical assembly rule: in a multispecies competition, species that all coexist with each other in pairs will survive, whereas species that are excluded by any of the surviving species will go extinct

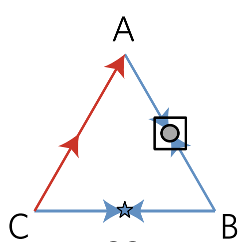


Friedman et al. Nature ecology & evolution 1, 0109 (2017).

Can we predict community behavior from pairwise interactions? Yes

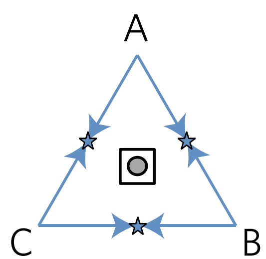
- All tri-cultures with 8 soil bacteria tested (CFU counts)
- Survival in 40 out of 56 correctly predicted with assembly rule

Example configurations:



22

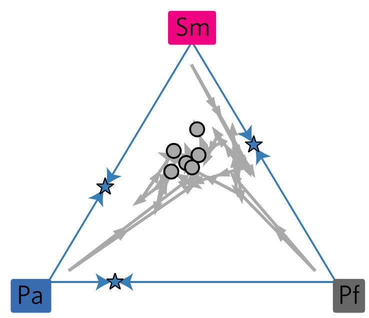
(out of 30)

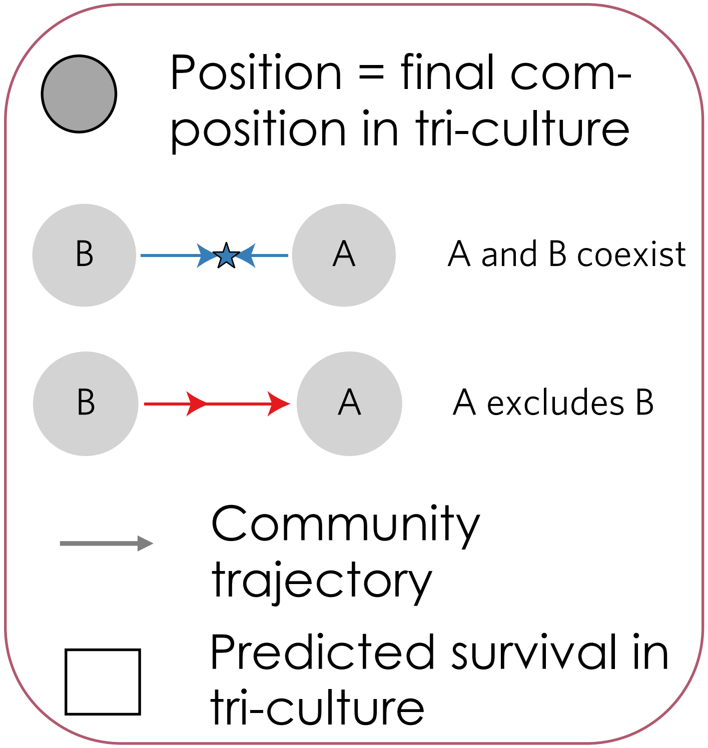


8

(out of 15)

Example case:





Friedman et al. Nature ecology & evolution 1, 0109 (2017).

Can we predict community behavior from pairwise interactions?

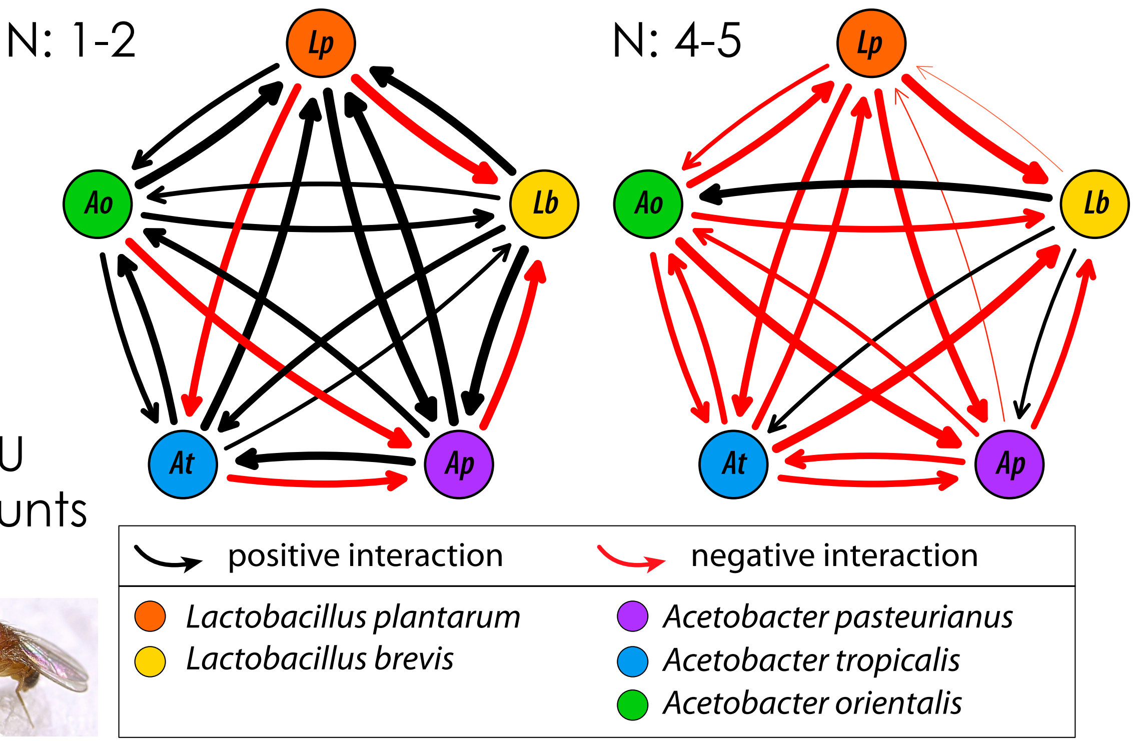
- *Drosophila melanogaster* is a good model host system:
  - Easy to keep and fast-growing
  - Eggs can be sterilized and larvae inoculated with desired bacteria via food
  - Only few gut microbial species
  - Gut species are easily culturable

Core gut bacteria:
Lactobacillus plantarum
Lactobacillus brevis
Acetobacter pasteurianus
Acetobacter tropicalis
Acetobacter orientalis

Gould et al. PNAS 115 (51), E11951-11960 (2018).

Can we predict community behavior from pairwise interactions? No

Interaction strengths in the presence of N species:



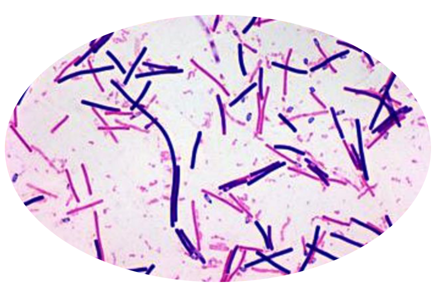
CFU counts

- Presence of other species alters interaction signs and strengths
- **Higher-order interactions** matter

Gould et al. PNAS 115 (51), E11951-11960 (2018).

Is it useful to look at pairwise interactions?

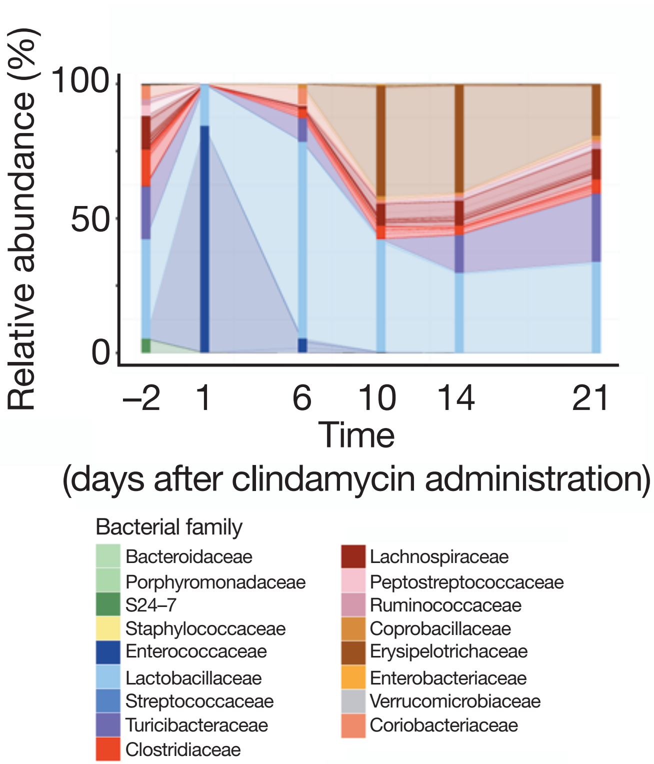
- *Clostridium difficile* is an intestinal pathogen in mammals
- It can thrive when killing gut microbiota with antibiotics
- Experiment: Mice infected with C. *difficile* after exposure to differen antibiotics

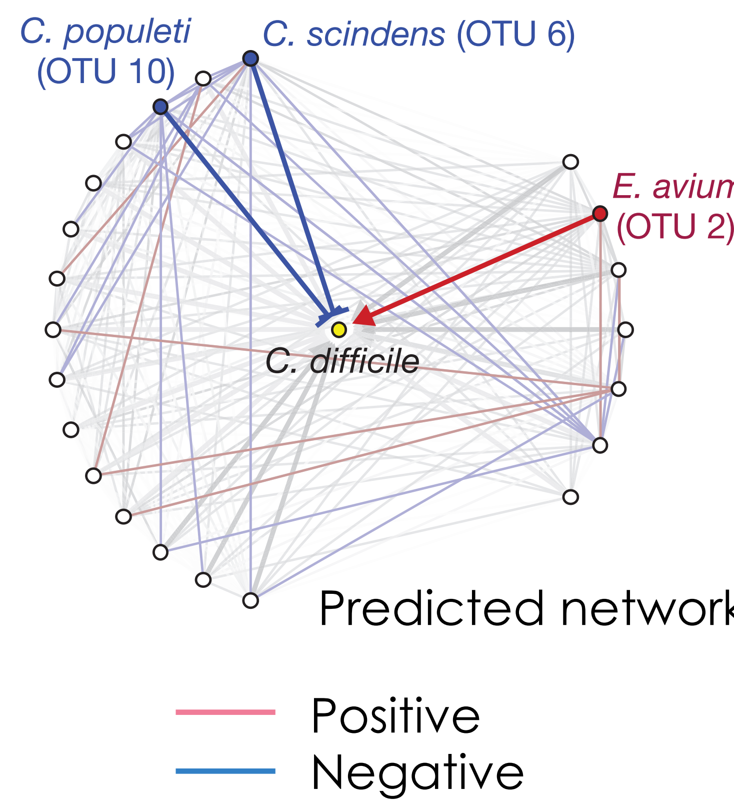


Buffie et al. Nature 517, 205-208 (2014).

Is it useful to look at pairwise interactions?

- Bacterial interaction network predicted from fecal microbial 16S time series of mice

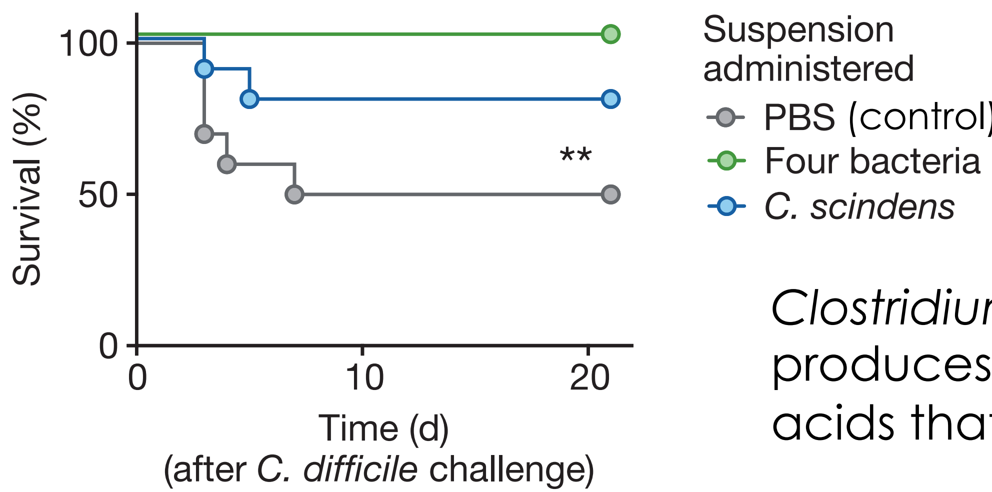




Buffie et al. Nature 517, 205-208 (2014).

Is it useful to look at pairwise interactions? Sometimes it is.

- Treating mice with bacteria that interact negatively with C. *difficile* increases their survival rate



Clostridium scindens produces secondary bile acids that inhibit C. *difficile*

Buffie et al. Nature 517, 205-208 (2014).

Summary part 2: community dynamics

- Community model: generalized Lotka-Volterra (gLV)
- GLV takes interaction matrix (= network) as input
- GLV assumes absence of higher-order interactions
- Co-occurrence analysis = network inference
- Network inference technique: significant covariance
- Microbial networks can predict ecological interactions
- Confounding factors exist: experimental validation is necessary
- Microbial networks can reveal niche structure
- Microbial networks predict keystone species with low accuracy; experimental validation is necessary

Take-home messages

- We can quantify microbial interaction strengths with mono- and co-cultures, but for this, we need to count species separately
- Co-culture dynamics can be hard to predict because microorganisms can change their metabolism in response to interaction partners
- Community behavior can be hard to predict because of higher-order interactions
- Interaction candidates can be predicted from community data with network inference
- Inferred interactions need to be experimentally validated

Thank you!

Appendix: Kinetic community model

Stefan Vet
Didier Gonze

Change of species abundances over time

$$\frac{dX_0}{dt} = \Gamma_0 \Phi_0(S_0, S_2) X_0$$

$$\frac{dX_1}{dt} = \Gamma_1 \Phi_1(S_0, S_x, S_2) X_1$$

$$\frac{dX_2}{dt} = \Gamma_2 \Phi_2(S_0, S_1) X_2$$

Growth functions

$$\Phi_0(S_0, S_2) = \mu_0 \frac{S_0}{K_{00} + S_0} \left(1 + \omega_0 \frac{S_2}{K_{02} + S_2}\right)$$

$$\Phi_1(S_0, S_x, S_2) = \mu_1 \frac{S_x}{K_{1x} + S_x} \frac{S_0}{K_{10} + S_0} \left(1 + \omega_1 \frac{S_2}{K_{12} + S_2}\right)$$

$$\Phi_2(S_0, S_1) = \mu_2 \left(\frac{S_0}{K_{20} + S_0} + \omega_2 \frac{S_1}{K_{21} + S_1}\right)$$

Change of substrate concentrations over time

$$\frac{dS_i}{dt} = -\sum_{j=0} v_{ij} \Phi_{ji} X_j$$

Lag phase function

$$\Gamma_i = \frac{Q_i}{1 + Q_i} \qquad \frac{dQ_i}{dt} = \mu_i Q_i$$

Species (X)
R. intestinalis
F. prausnitzii
B. hydrogenotrophica

Substrates (S)
Fructose
Acetate
Unknown compound
Formate

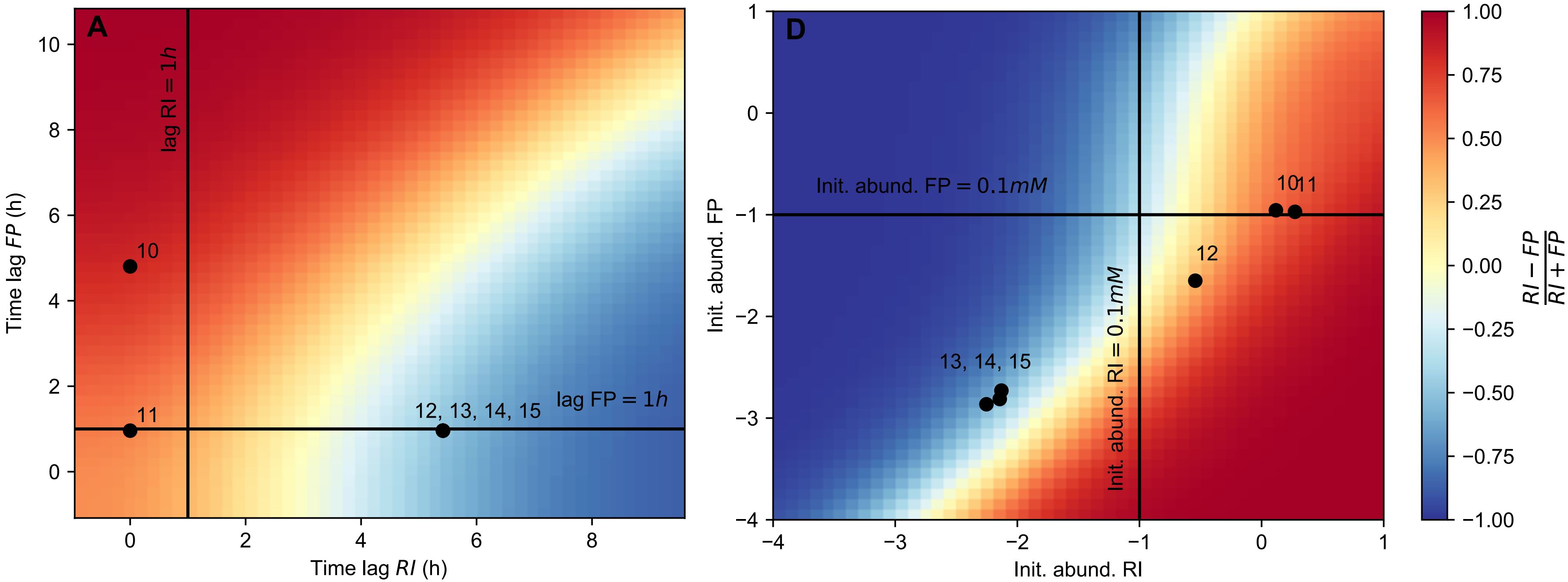
Constants

μ_i: max growth rate of species i
ω_i: nutrient weight of species i
Q_i: lag phase variable of species i

v_{ij}: production/consumption rate of metabolite i by species j
K_{ij}: Monod constant of species i for metabolite j

Appendix: Community model parameterized with mono- and bi-cultures fits tri-culture well

- Final abundance ratio for RI and FP predicted with the model agrees with experimental observations



Lag phase varied; initial abundances kept constant

Initial abundances varied (log scale), lag phase kept constant (final abundance ratio in experiment 12 deviates from prediction)