Phage vs Bacteria: The art of war among the unseen majority

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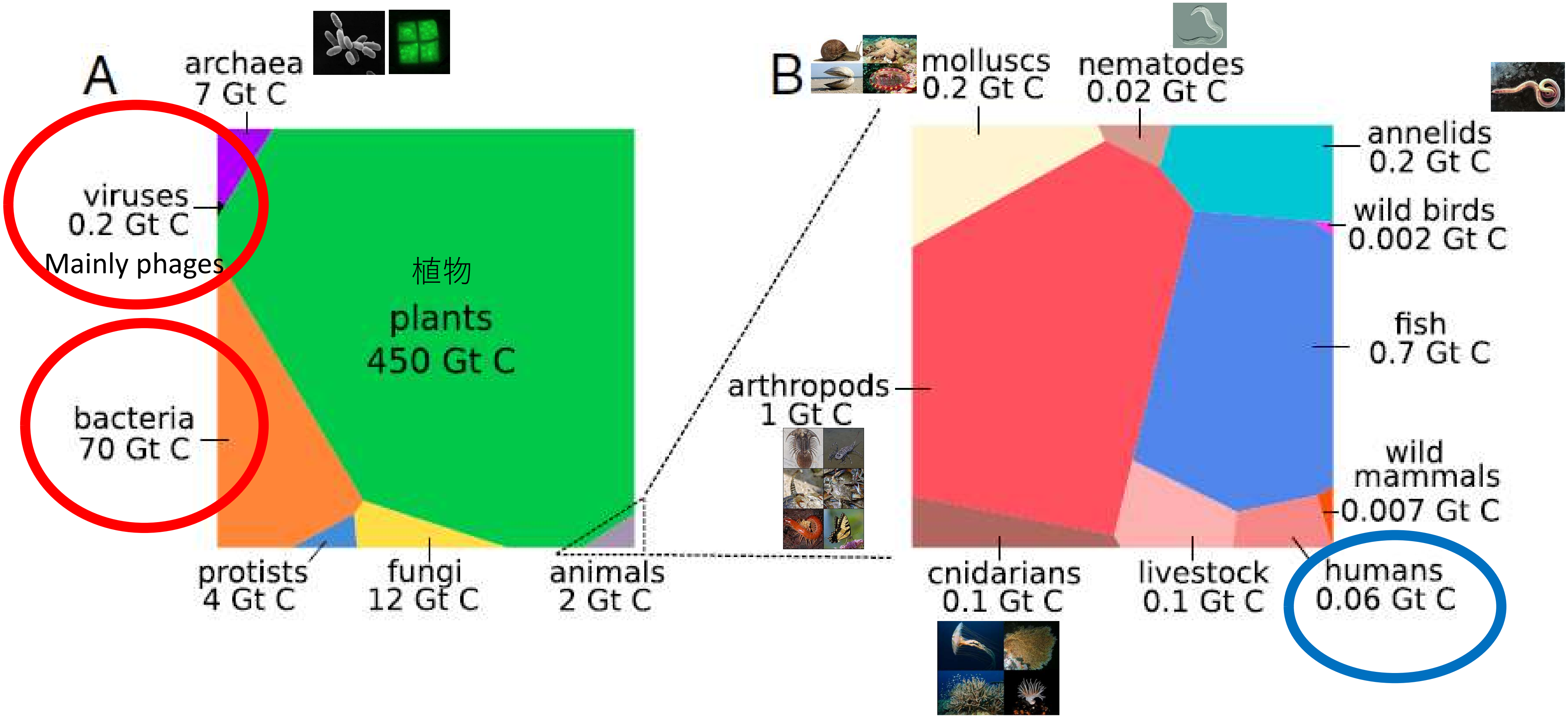
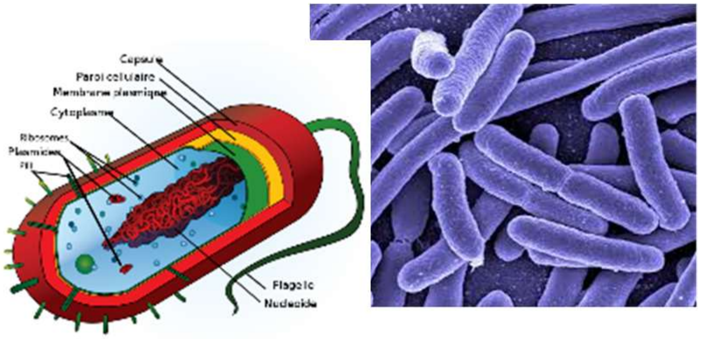
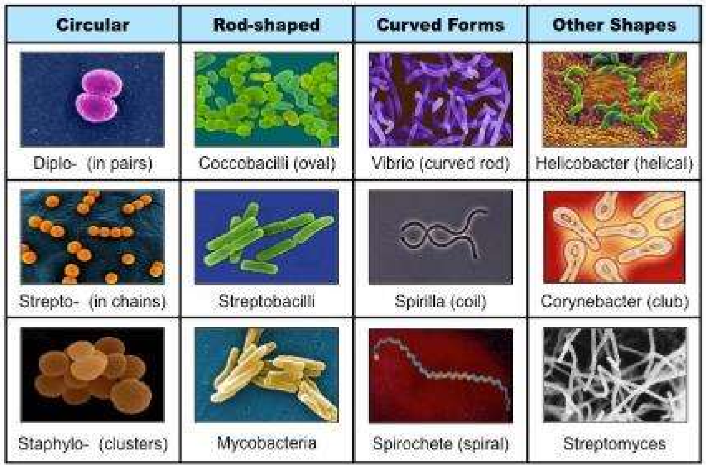
Graphiscs by Hannah Heilmann (J Virol. 2010 Apr; 84(8): Cover)

Estimate of Biomass on earth in Gigatons of Carbon
(from Yinon et al. PNAS 2018)



Bacteria: The unseen majority





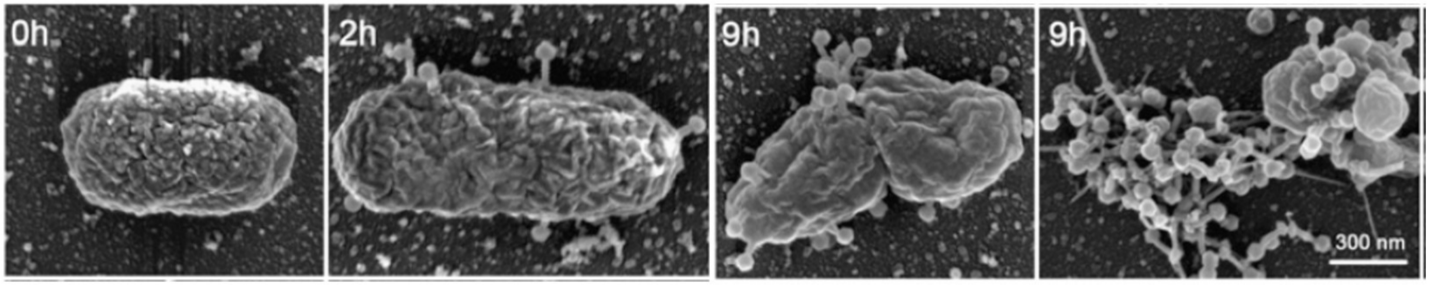
From: http://ib.bioninja.com.au/standard-level/topic-1-cell-biology/12-ultrastructure-of-cells/types-of-bacteria.html

- ~1 μm^3 scale in size
- ~ 10^6 per ml of fresh water
- ~ 5×10^{30} bacteria cells on earth (Whitman et al. 1998)
    - # of cells in a human body: 3×10^{13}
- Exponential growth when happy: a good model system

Stewart EJ et al (2005). PLoS Biol. 3 (2): e45

(Bacterio)phage: Nemesis of bacteria

Phage = viruses that infect bacteria



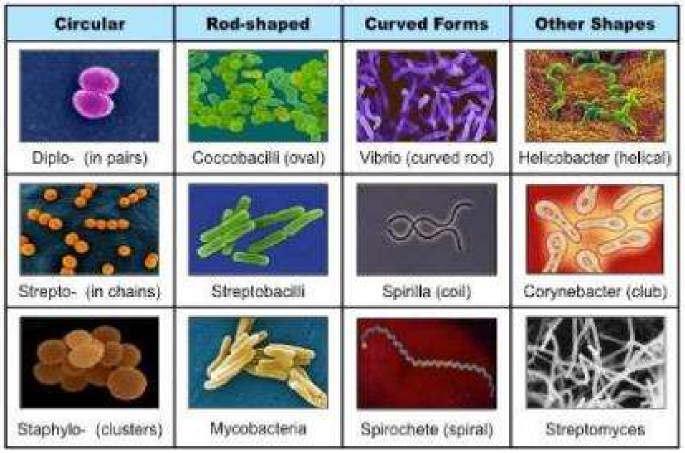
From: Sabehi et al PNAS 109, 2037-2042 (2012)

Infecting one bacterium -> >100 of phages come out

Phages shape microbial ecology and evolution

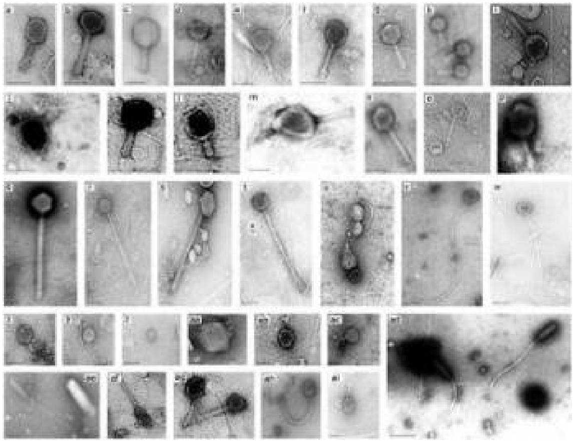
~ 5×10^{30} bacteria cells on earth
(Whitman et al. 1998)

~ phage to bacteria ratio: 3 to 10
(Wommack and Colwell 2000)



From https://ib.bioninja.com.au/standard-level/topic-1-cell-biology/12-ultrastructure-of-cells/types-of-bacteria.html

VS

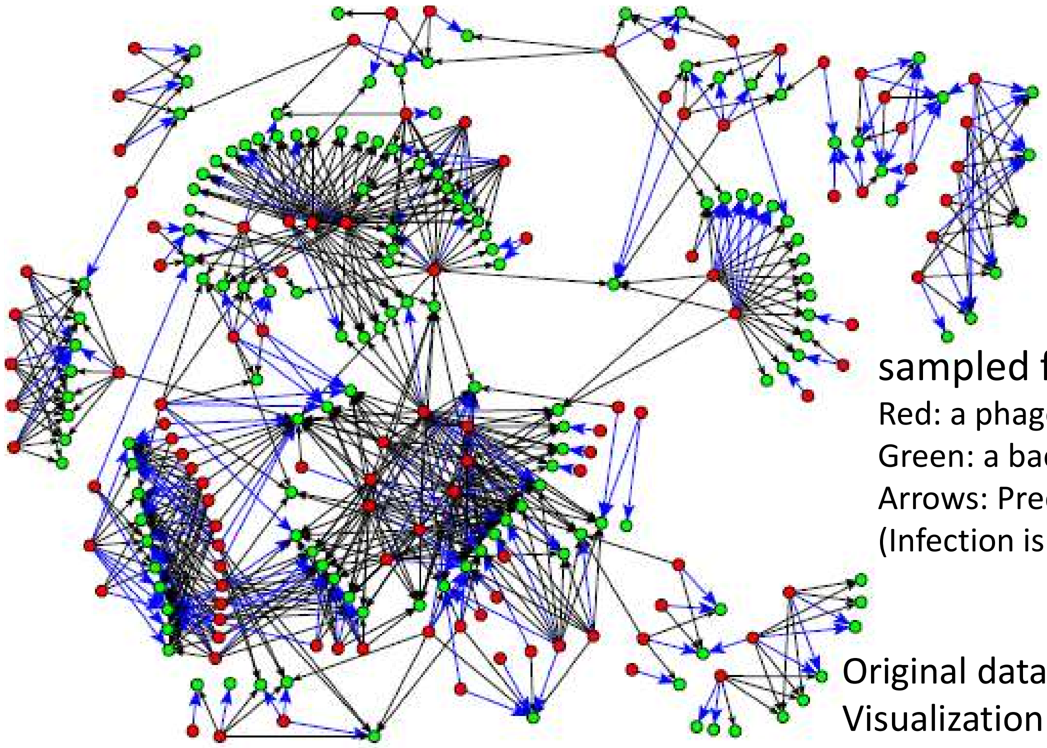


From Sulcius et al. Oceanologia (2011)

- Microorganisms consists 90% of biomass in ocean, 20% of them killed by virus every day (Suttle Nat. Rev. Microbiol. 2007)

If you find life on another planet it can be bacteria and phage like!

High diversity in Bacteria-phage ecosystem: Many interact and coexist



sampled from Atlantic ocean

Red: a phage type

Green: a bacteria type

Arrows: Predator-Prey relation

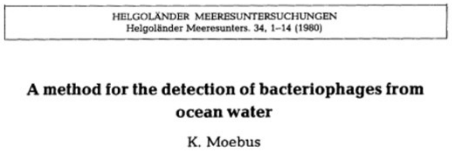
(Infection is specific)

Original data from Moebus (1980)

Visualization by K. Sneppen and S. Maslov

Data from Flores et al (2012)

..Or ?



HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 34, 1–14 (1980)

A method for the detection of bacteriophages from ocean water

K. Moebus

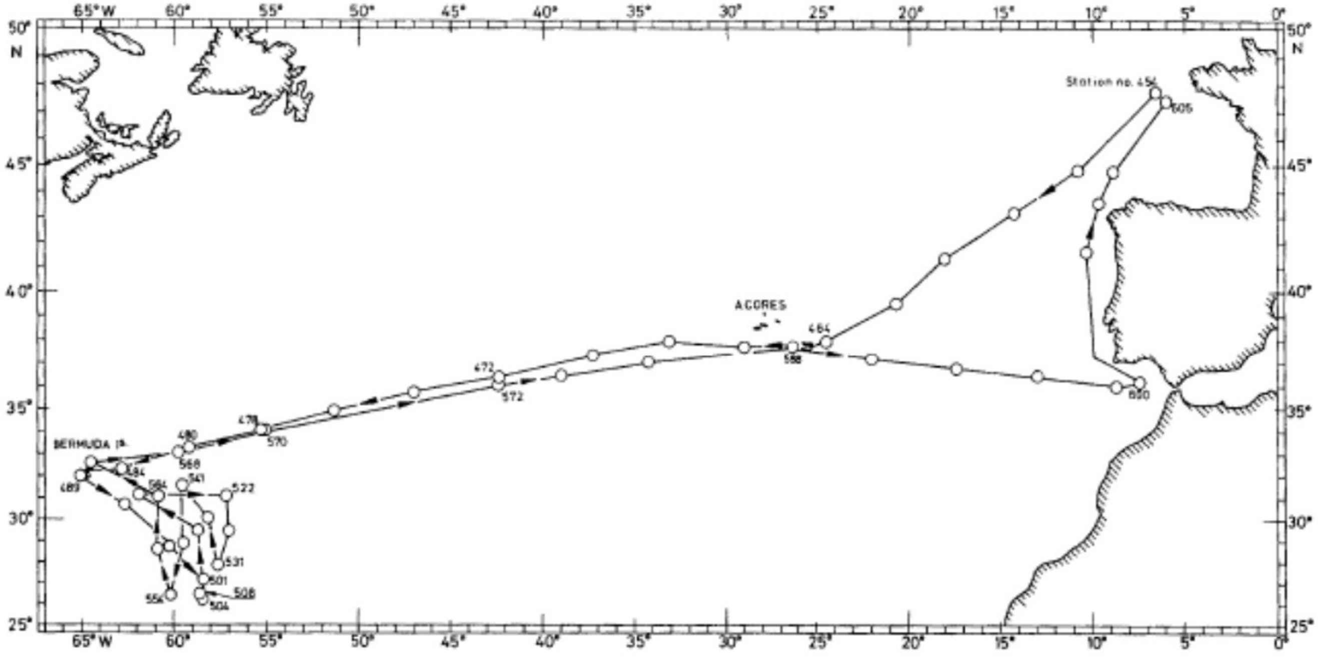
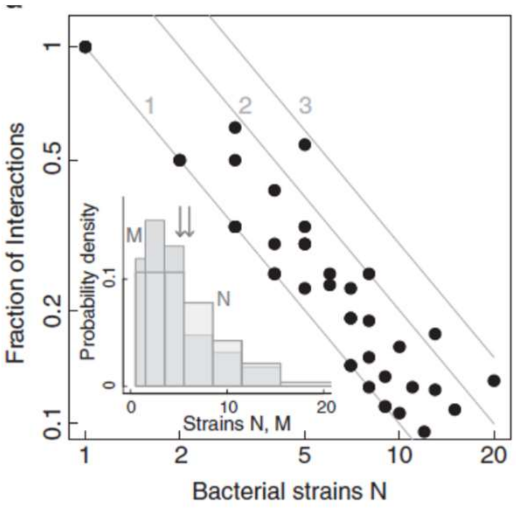


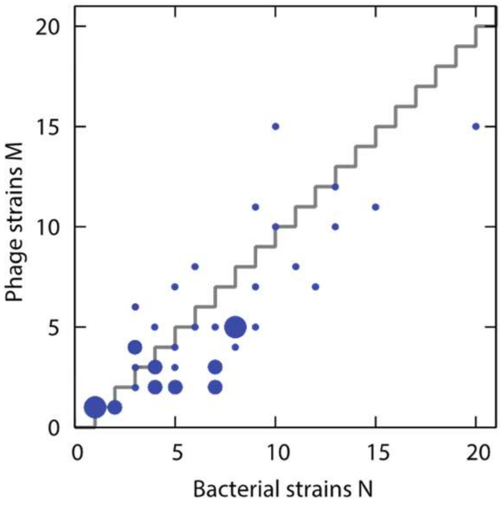
Fig. 1. Track of RV "Friedrich Heincke" in the Atlantic Ocean during cruise no. 160 and microbiological stations

Separate data per station

Infection :
1phage type – 1 bacteria type



Bacteria type N~ phage type M,
N, M<15



Haerter, NM, Sneppen, ISME (2014)

Let’s try a Lotoka-Volterra eq. with logistic growth (Well-mixed model)

Bacteria:

$$\frac{dB}{dt} = \underline{kB\left(1-B\right)} - \underline{\alpha B} - \boxed{\eta BP}$$

Logistic growth
normalized to max. population
(~10^7 /ml in ocean)

“Natural
death”

Infected by phage

Phage:

$$\frac{dP}{dt} = \boxed{\beta\eta BP} - \underline{\delta P}$$

burst size β ~ 100

“Natural
death”

Let’s try a Lotoka-Volterra eq. with logistic growth (Well-mixed model)

Bacteria:

$$\frac{dB}{dt} = \underline{kB(1-B)} - \underline{\alpha B} - \boxed{\eta BP} \quad = B[k(1-B) - \alpha - \eta P]$$

Logistic growth
normalized to max. population
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Infected by phage

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$$\frac{dP}{dt} = \boxed{\beta \eta BP} - \underline{\delta P} \quad = P[\beta\eta B - \delta]$$

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burst size β ~ 100

“Natural
death”

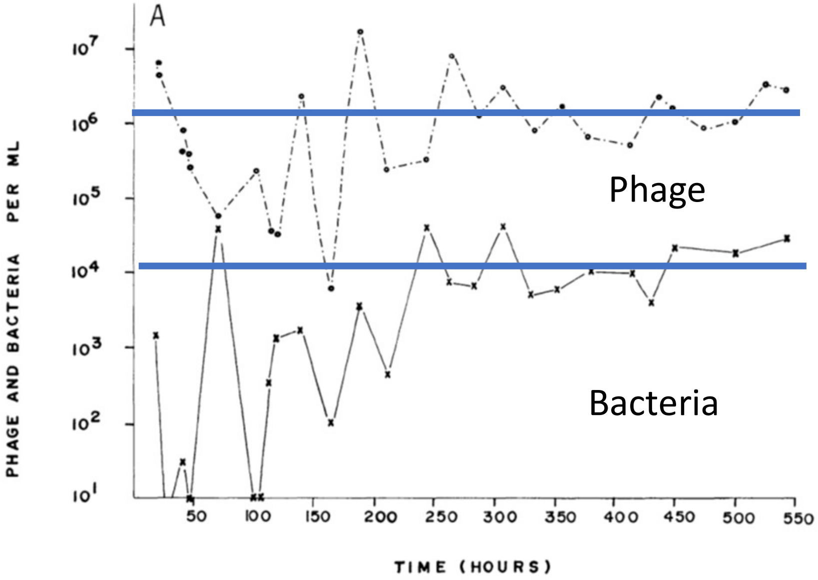
Steady state solutions:

$$B = 0, P = 0$$

$$B = 1 - \frac{\alpha}{k}, P = 0$$

$$B = \frac{\delta}{\beta\eta}, P = \frac{1}{\eta}\left[k\left(1 - \frac{\delta}{\beta\eta}\right) - \alpha\right]$$

Phage and bacteria can coexist
A. Campbell (1961)



$$P = \frac{1}{\eta}\left[k\left(1-\frac{\delta}{\beta\eta}\right)-\alpha\right]$$

$$B = \frac{\delta}{\beta\eta}$$

(experiment: B. Levin et al 1977)

Extend to multiple types system

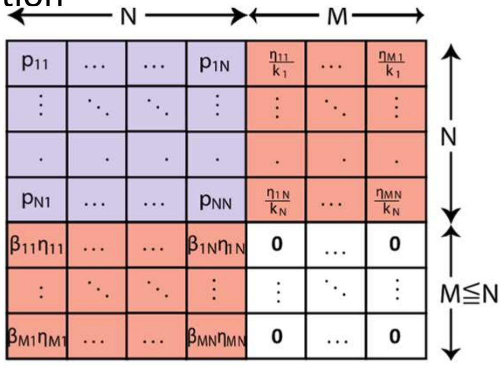
$$\frac{dB_i}{dt} = B_i\left[k_i\left(1 - \sum_{j=1}^{N} p_{ji}B_j\right) - \alpha_i - \sum_{k=1}^{M}\eta_{ki}P_k\right]$$

$$\frac{dP_k}{dt} = P_k\left[\sum_{m=1}^{M}\beta_{km}\eta_{km}B_m - \delta_k\right]$$

Linear in B_i's and P_k's

Non-zero steady state solution

$$S = \begin{pmatrix} B_1 \\ \vdots \\ B_N \\ P_1 \\ \vdots \\ P_M \end{pmatrix}, \quad \mathcal{R} \cdot \mathbf{S} = \mathbf{k}$$



$$R = \begin{pmatrix} p_{11} & \cdots & \cdots & p_{1N} & \frac{\eta_{11}}{k_1} & \cdots & \frac{\eta_{M1}}{k_1} \\ \vdots & \ddots & \ddots & \vdots & \vdots & \ddots & \vdots \\ . & . & . & . & . & . & . \\ p_{N1} & \cdots & \cdots & p_{NN} & \frac{\eta_{1N}}{k_N} & \cdots & \frac{\eta_{MN}}{k_N} \\ \beta_{11}\eta_{11} & \cdots & \cdots & \beta_{1N}\eta_{1N} & 0 & \cdots & 0 \\ \vdots & \ddots & \ddots & \vdots & \vdots & \ddots & \vdots \\ \beta_{M1}\eta_{M1} & \cdots & \cdots & \beta_{MN}\eta_{MN} & 0 & \cdots & 0 \end{pmatrix}$$

Condition to
have a solution

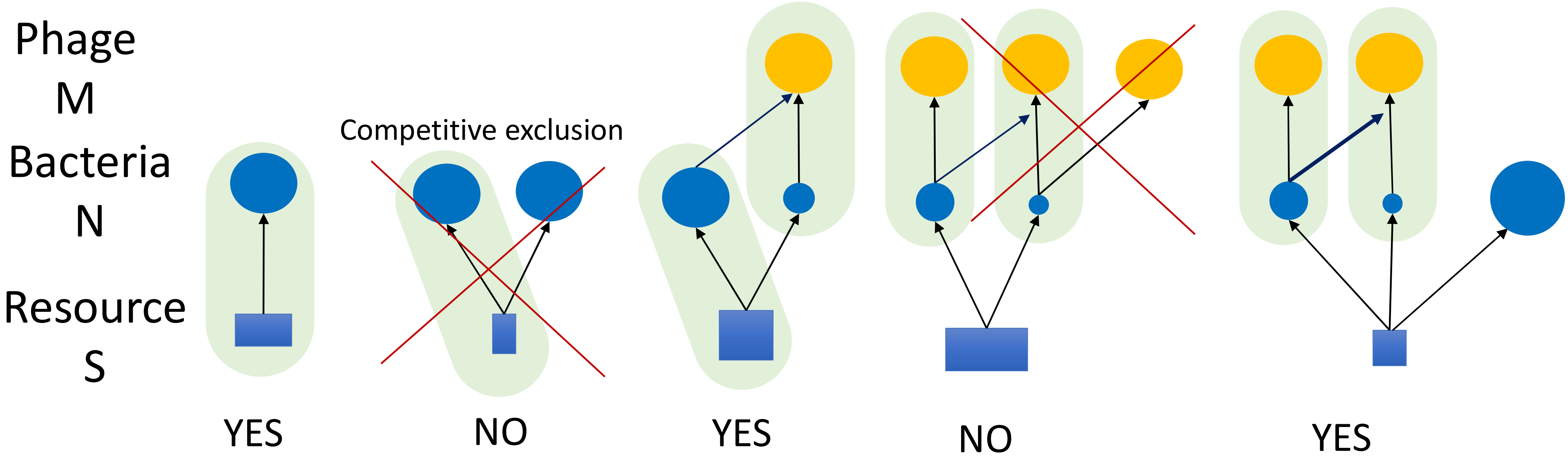
$$det(\mathcal{R}) \neq 0$$

Haerter, NM,Sneppen, ISME (2014)

Haerter, NM,Sneppen, ISME (2014)

Constraint predicted by the model: Non-overlapping paring rule

cf. Competitive exclusion (Gause 1934, Hardin 1960)



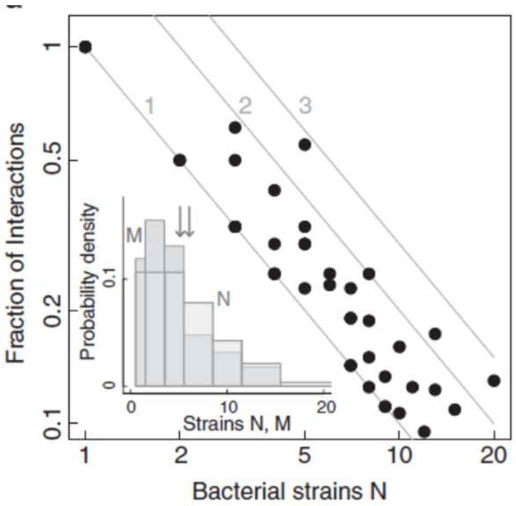
- N=M or N=M+1 or …. N=M+S
- Simplest interaction network: 1 phage strain – 1 bacteria strain (diagonal)

Levin SA (1970): # of coexisting species cannot be greater than # of distinct regulating factors in the community.
Extended to multi-trophic level foodweb (Haeter, NM, Sneppen, Plos Comput. Biol. 2016)

The model consistent with the station separated data

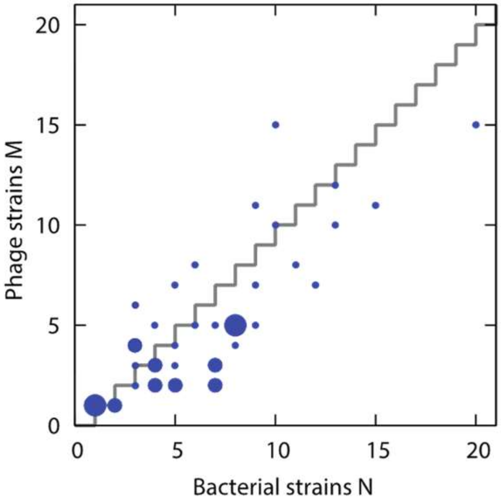
Infection: ~1 phage type – 1 bacteria type

diagonal infection network +



Bacteria type N~ phage type M, N, M<15

Climbing the narrowing stairs



Haerter, NM, Sneppen, ISME (2014)

What if the system is NOT well-mixed?
- for example CHEESE?

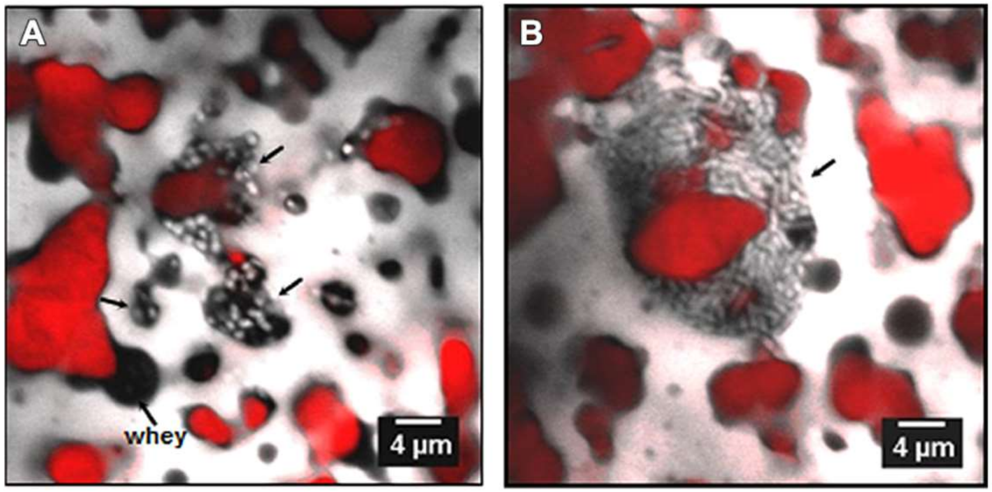
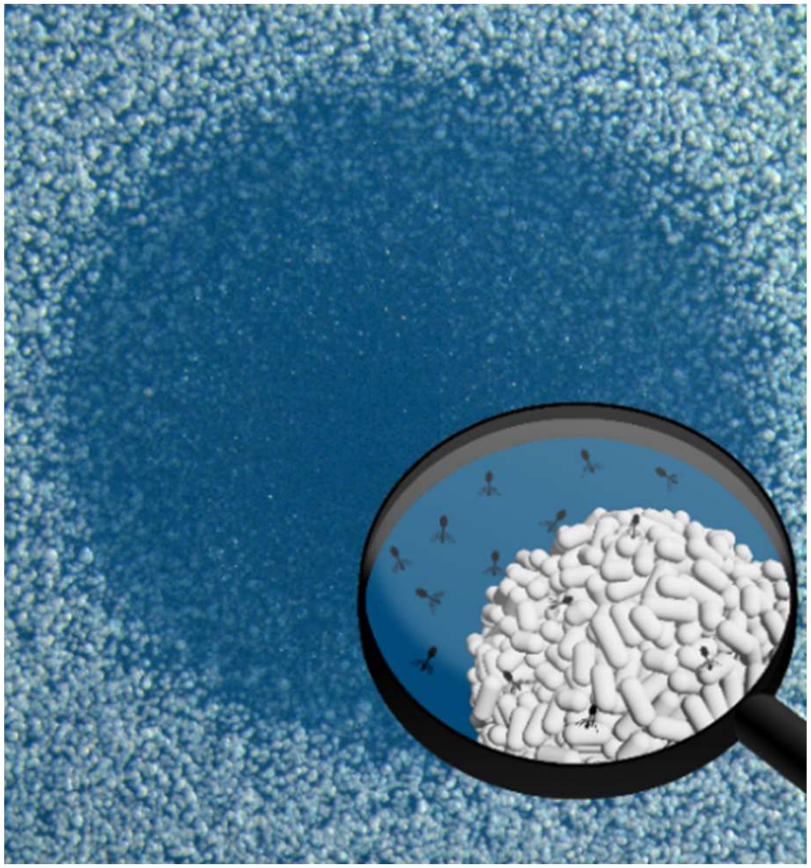


FIGURE 6 | Confocal laser scanning microscopy images of bacteria in Emmenal cheese after 1 day of ripening, showing location of bacterial colonies (light color) in whey pockets (A, black areas) and at the interface (B) between fat (red) and protein (gray). Adapted from Lopez et al. (2006) with permission from the authors.

From Hickey, C.D., Sheehan, J.J., Wilkinson, M.G. and Auty, M.A., 2015. *Frontiers in microbiology*, *6*, p.99.
(Original Image from Lopez et al. J. Agric. Food Chem. 2006, 54, 5855−5867)

Phage attack on a colony?

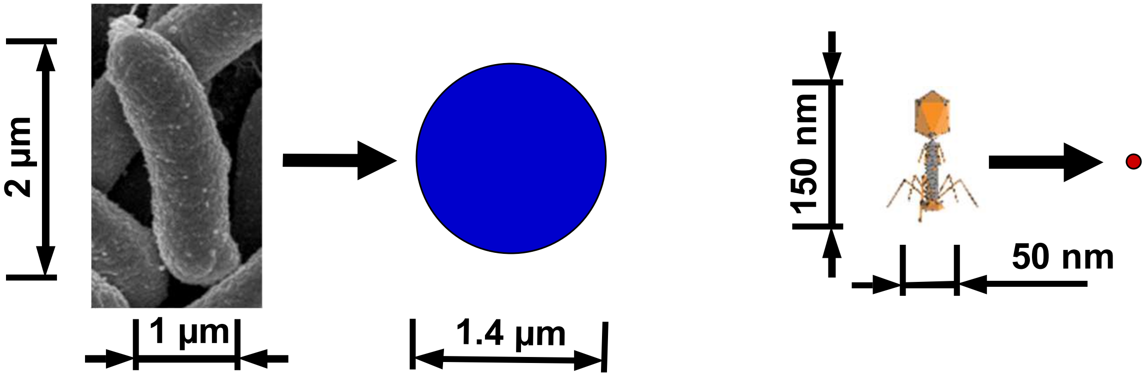


Simulation by Rasmus Eriksen

Simulations

Agent based simulation

→ Every cell and phage modeled individually



Eriksen, Svenningsen, Sneppen, NM, PNAS (2018)

Exponential cell growth to form a spherical microcolony

Exponentially growing Cells

In soft agar, 3D spherical colony is reported to grow exponentially up to ~100 μm in diameter
(Shao et al. Plos. Compt. Biol. 2017)

Eriksen, Svenningsen, Sneppen, NM, PNAS (2018)

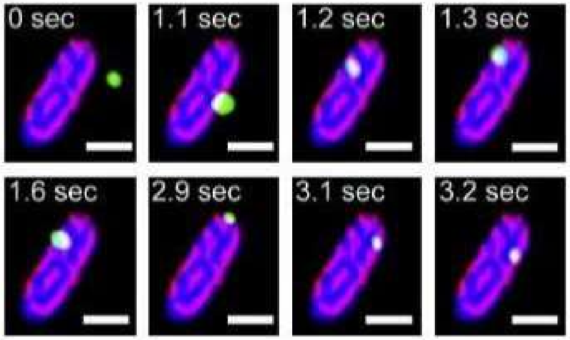
Infection by phage

A Phage Diffuses, recognizes A surface receptor, and injects its DNA

Diffusion Limited infection

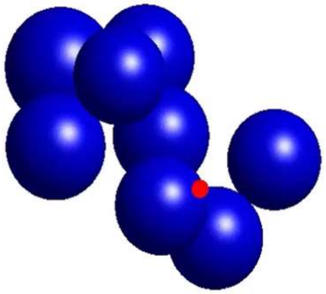
(High receptor number limit)

Single phage tracking:
Rothenberg et al. Biophys. J. (2011)



Green: A phage particle
Purple: Receptors
Scale bar: 2μm

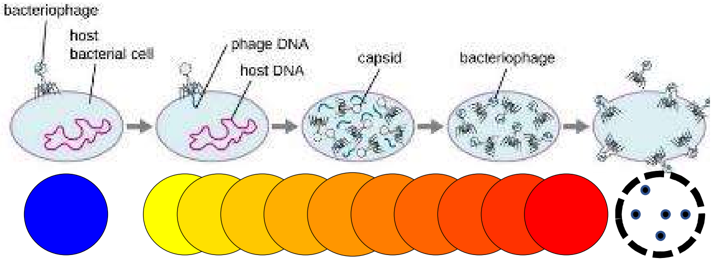
Image by R. Hendrix
https://www.asm.org/division/m/foto/LamAttack.html



- A phage: A point particle, Brownian motion
- Infection: When it comes “inside” a cell
- **Phage cannot tell if a cell is already infected or not: Superinfections allowed**

Eriksen, Svenningsen, Sneppen, NM, PNAS (2018)

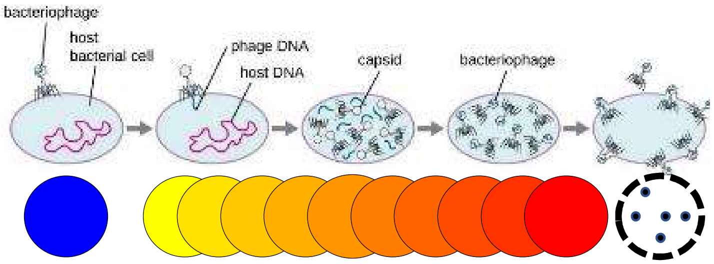
Phage attack on a growing colony: Simulation



400 phages born at every burst
A phage diffuses and infect new bacteria, but now shown in the movie

Eriksen, Svenningsen, Sneppen, NM, PNAS (2018)

2D killing vs 3D growth =Critical size

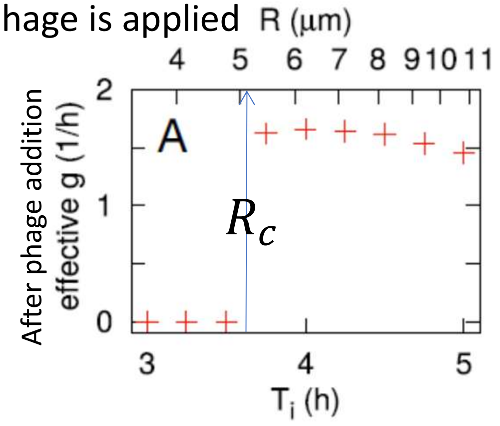


- **Most of them superinfect already infected cells: killing limited to surface**

Eriksen, Svenningsen, Sneppen, NM, PNAS (2018)

3D Growth vs 2D killing = Critical size

Size of colony when the phage is applied



$$\frac{dV(t)}{dt} = \frac{4}{3}\pi\left[g(R(t)-\Delta R)^3 - \frac{1}{\tau_L}(R(t)^3 - (R(t)-\Delta R)^3)\right]$$

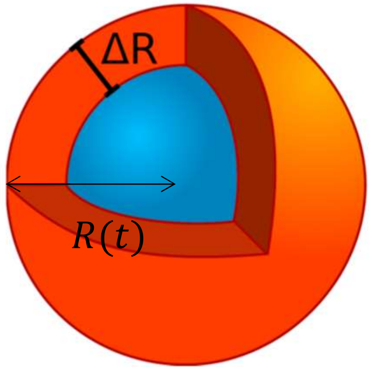
Critical size (dV/dt=0):

Phage penetration depth

Phage latency time

$$R_c \approx 3\left(1+\frac{1/\tau_L}{g}\right)\Delta R$$

Bacterial growth rate

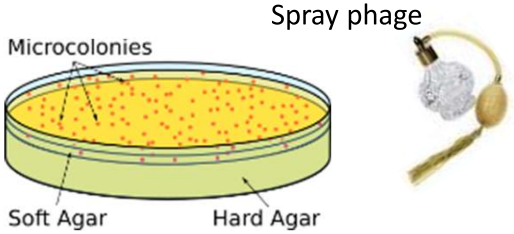


(In small penetration depth limit. Full eq. can be analytically solved.)

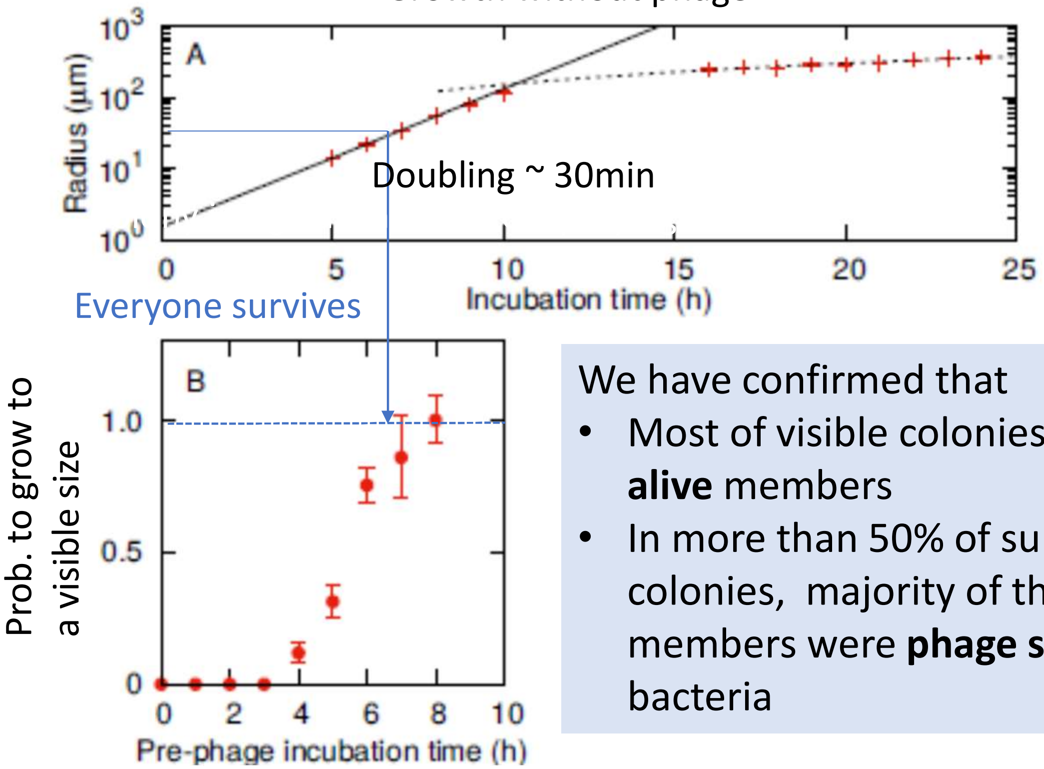
Eriksen, Svenningsen, Sneppen, NM, PNAS (2018)

Experiment: Transition from death to survival

Rasmus Eriksen &
Sine Svenningsen

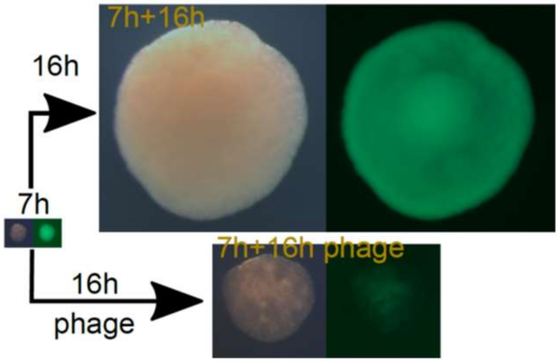


Growth without phage



Doubling ~ 30min

Everyone survives



We have confirmed that

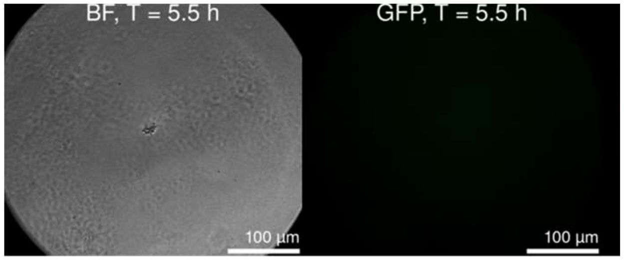
- Most of visible colonies contained **alive** members
- In more than 50% of surviving colonies, majority of the surviving members were **phage sensitive** bacteria

Eriksen, Svenningsen, Sneppen, NM, PNAS (2018)

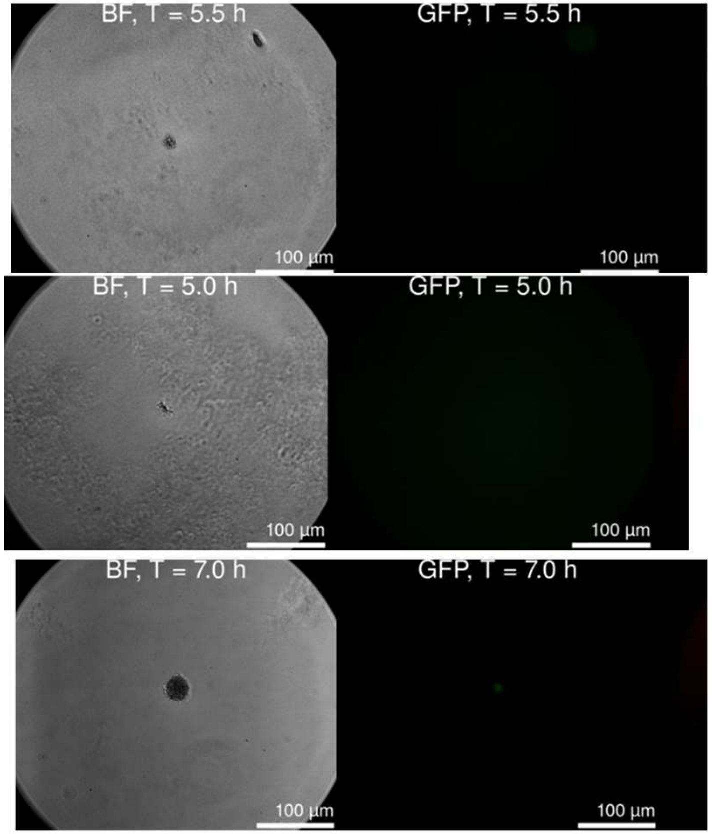
Sneak peak: Dynamics of colony under attack of virulent phage T4

Eriksen & Larsen

No phage



With phage



Eriksen, Larsen, Svenningsen, NM, Sneppen, in preperation

Lesson: Space matters from km to <mm
We need to know the needed resolution

- Phage-bacteria interaction in ocean
  - Separating data to local stations consistent with well-mixed model prediction
- Geometry of just growing together results in active coexistence
  - Relevant in various spatially structured situation: biofilm, soil, even in the ocean?
  - Agent based model naturally reproduces the protection by superinfection by having geometry and the discreteness of the entities

- Competitive exclusion and coexistence: J. Haerter, NM, K. Sneppen, ISME J (2014)
  - Follow-up for foodweb: J. Haerter, NM, K. Sneppen, Plos Comp (2016); PRE (2018)
  - Follow-up for evolution of ecosystem with cross links: A. Marantos, NM, K. Sneppen, Plos Comp e1010400 (2022)
- Colony attack: R. Eriksen, S. L. Svenningsen, K. Sneppen, NM, PNAS **115**, 337 (2018)
  - Experiment on the time-course: Eriksen, Larsen, Svenningsen, NM, Sneppen, in preparation
- Hybrid model: R. Eriksen, NM, K. Sneppen, Sci.Rep. (2020)