Bacteriophages: from enemies to allies of dairy plants

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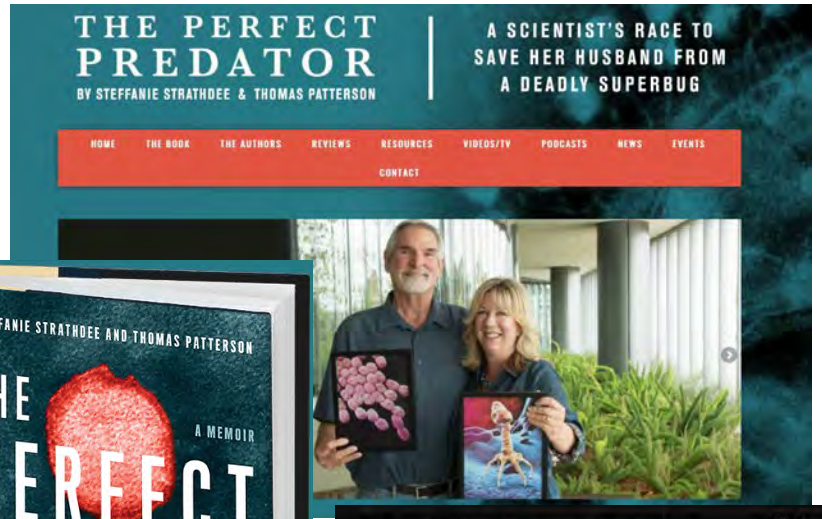
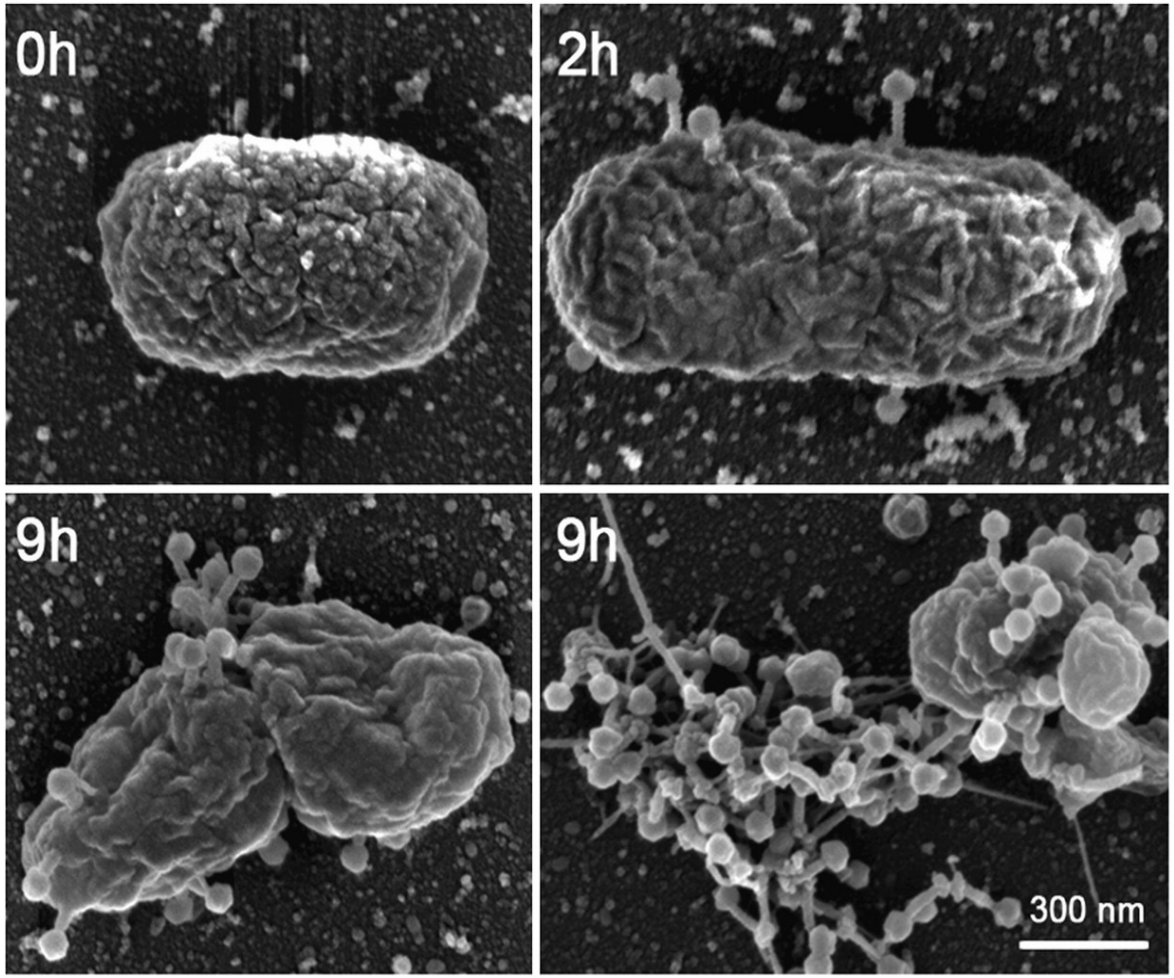
Bacteriophages as alternative to antimicrobials

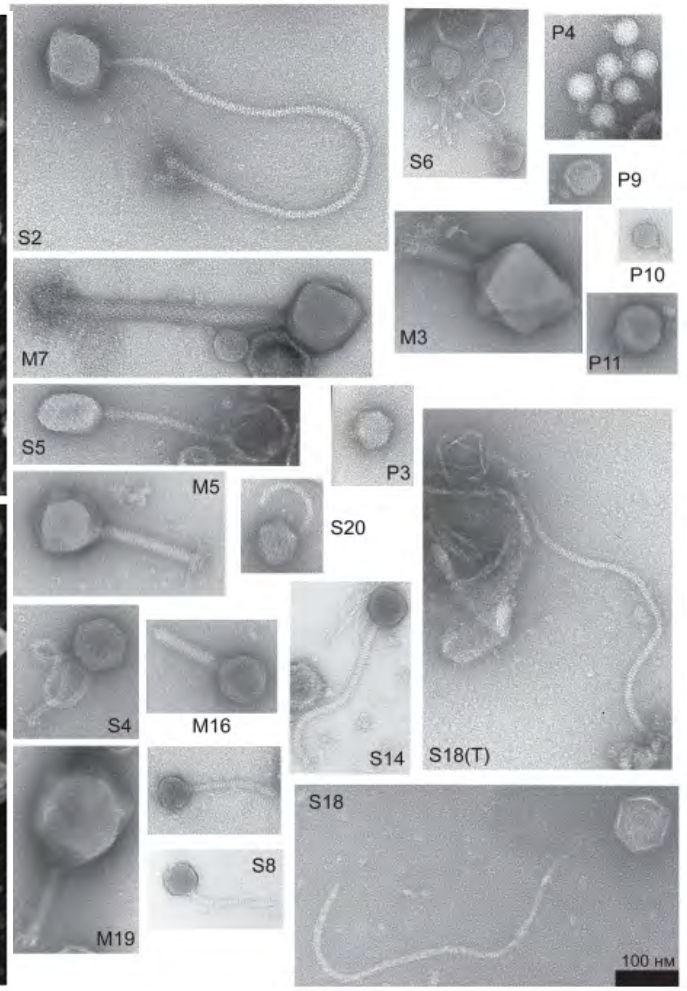
PHAGE THERAPY against human pathogens



PHAGE BIOCONTROL in food and primary production, against crop disease, in livestock farming and aquaculture, surface sanitizing, food preservatives,...

Bacteriophages are the natural predators of bacteria





specifically infect and kill bacteria by cell lysis

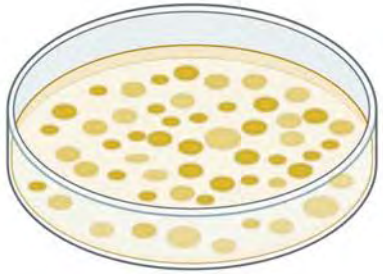
10 times smaller than bacteria, but 10 times more abundant and impact on microbial ecosystems

same places where bacteria are

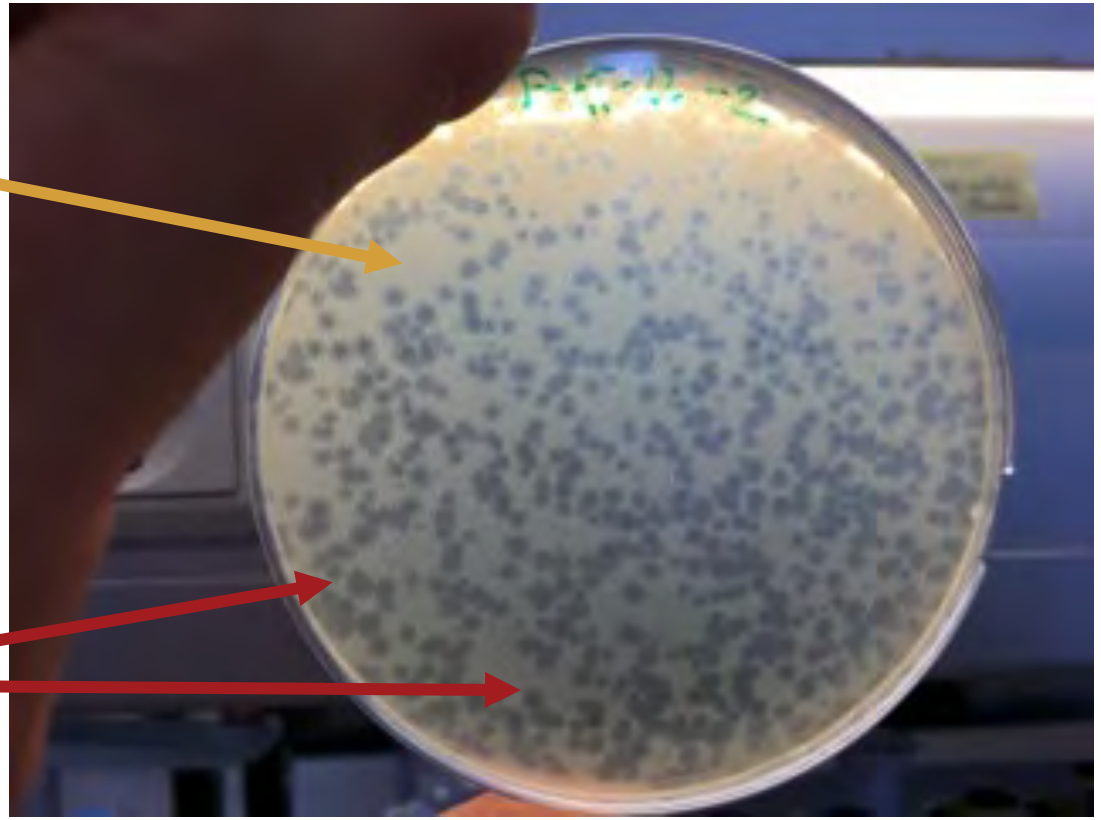
Kulikov et al. 2007; Sabehi et al. 2012

Bacteriophages killing bacteria in the laboratory

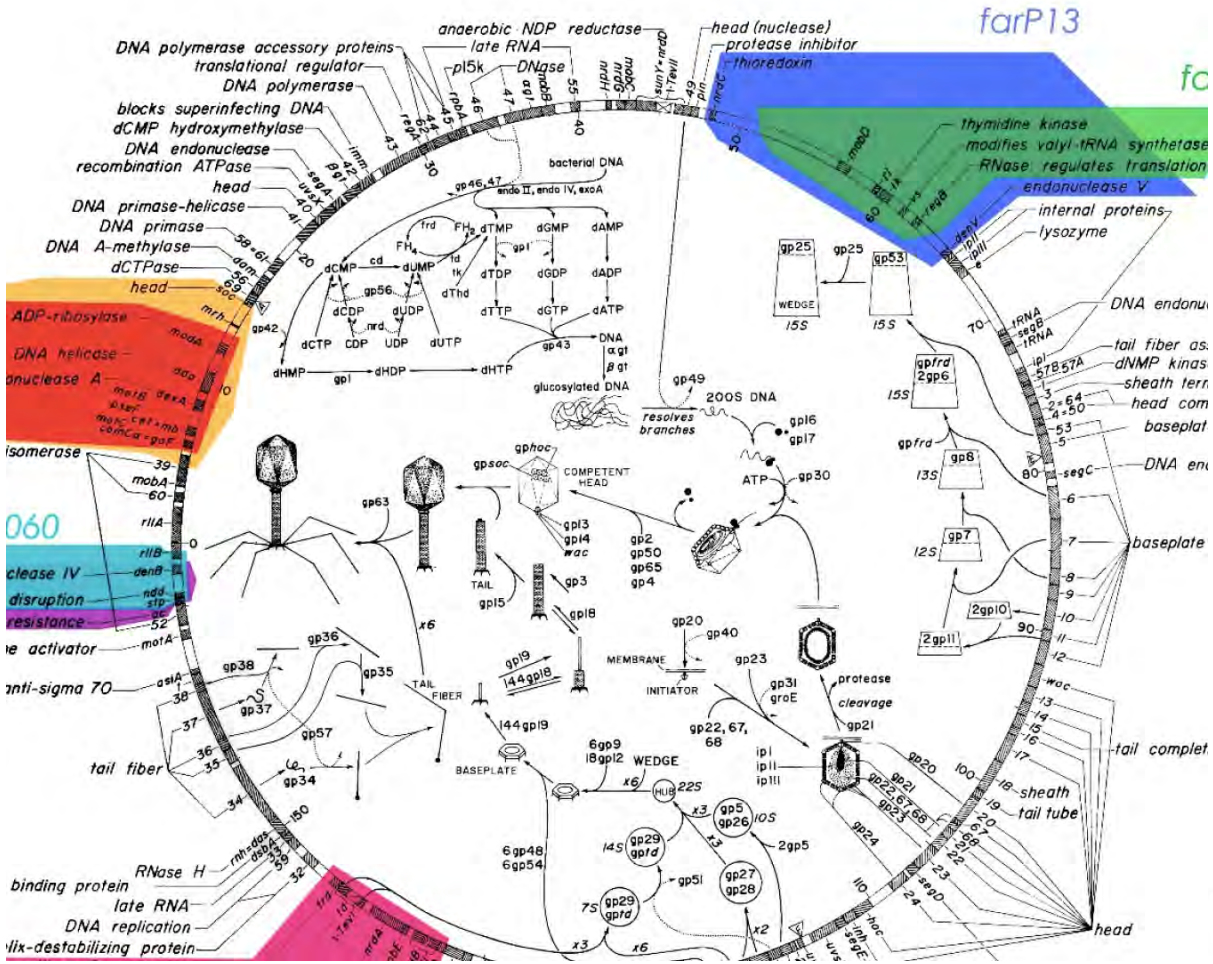
Lawn of bacteria in soft agar



Plaques



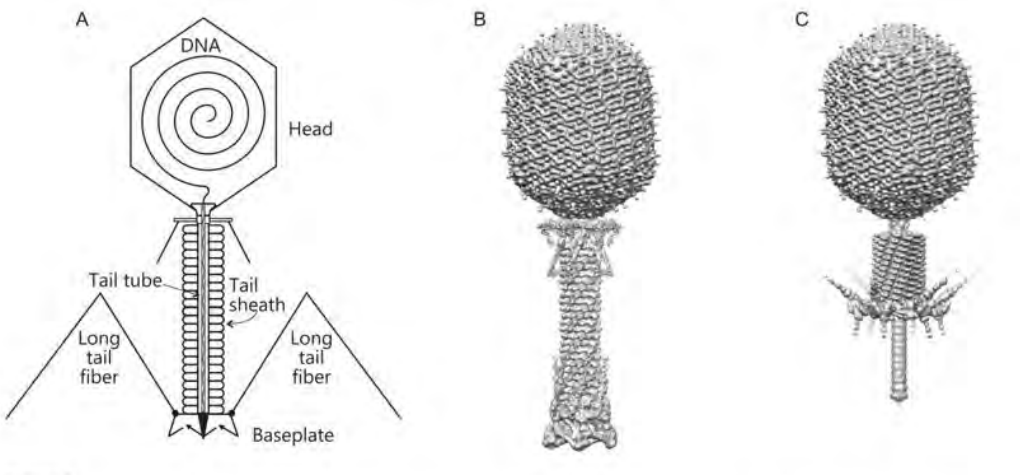
Bacteriophages are made of DNA and proteins



Kutter et al. 2018; Leiman et al. 2010

Details about the function of genes/proteins from

- advanced microscopy
- DNA sequencing
- omics
- engineering of phages and bacteria

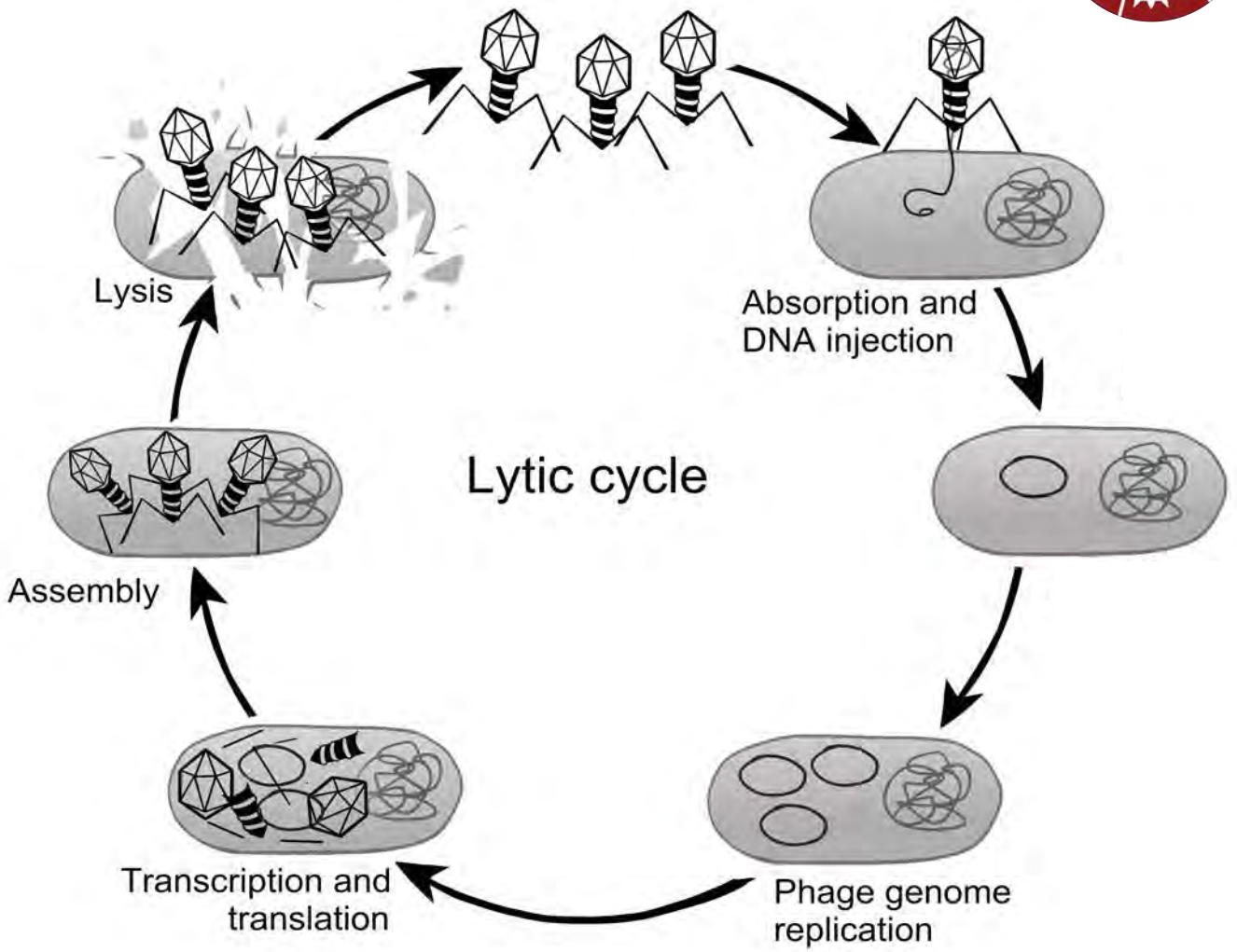


Bacteriophages are specific for their bacterial host

Infection and propagation require high compatibility between bacteriophage and host

Each phage has a specific host or few hosts that it can infect, kill and replicate in

- phages infecting only specific *E.coli* strains → ☺ but also a limitation
- a phage infecting *E.coli* cannot infect *Staphylococcus* spp. → ☺

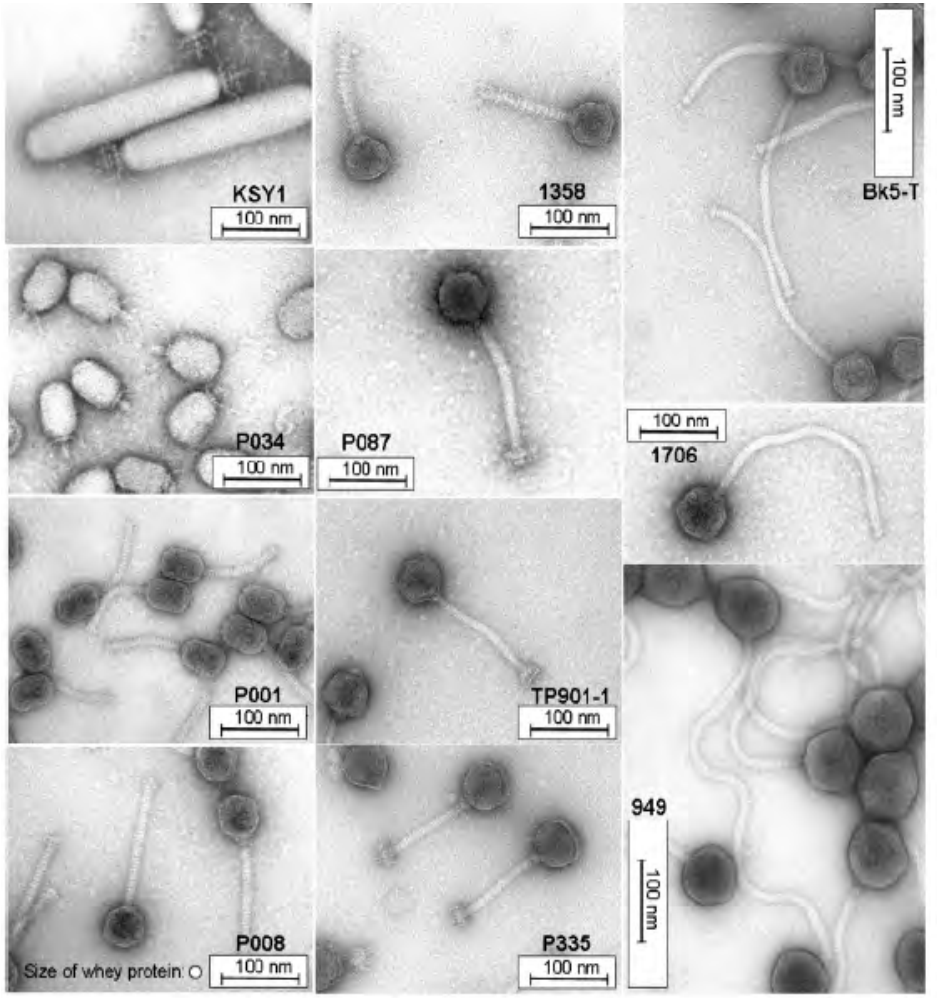


Bacteriophages in the dairy sector

ENEMIES
- phages infecting Lactic Acid Bacteria (LAB)
- fermentation failure - spoilage or delay of dairy products → large economic loss

Phages disseminate and persist on surfaces

Control measures:
- physical: heating, filtration, high pressure, UV, electro-impulse treatments
- chemical: biocides
- biological: strain rotation, use of strains with improved phage resistance



Atamer et al. 2013

Can bacteriophages help the dairy industry?

Renessaince of phage biology: key recent developments and discoveries to understand infection mechanisms and specificity

1) to ensure food safety → **improve the safety of powder infant formula**

2) increasing the sustainability of dairy process → **biofilm removal from dairy filtration systems**

Cronobacter sakazakii in powdered infant formula

- *Cronobacter sakazakii* is an opportunistic pathogen of the family of *Enterobacteriaceae*
- Disease in infants, with higher risk for premature infants: necrotizing enterocolitis, bacteremia, and meningitis
- It kills up to 40 percent of infected infants, the survivers face long-term neurological problems (Centers for Disease Control and Prevention)
- Many outbreaks traced to contaminated powdered infant formula

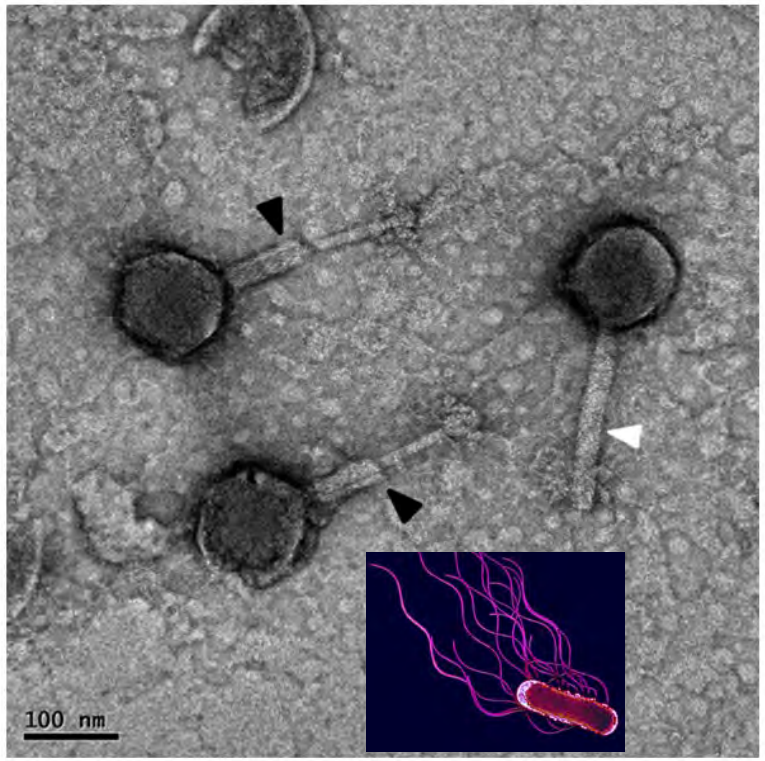
C. sakazakii causes major economic consequences

- Powdered infant formula is not sterile, so contamination possible at manifacture, but also during storage and reconstitution
- *C. sakazakii* tolerates dry conditions for long time, resists to heat, UV, reactive oxygen species, stomach acids and pasteurization

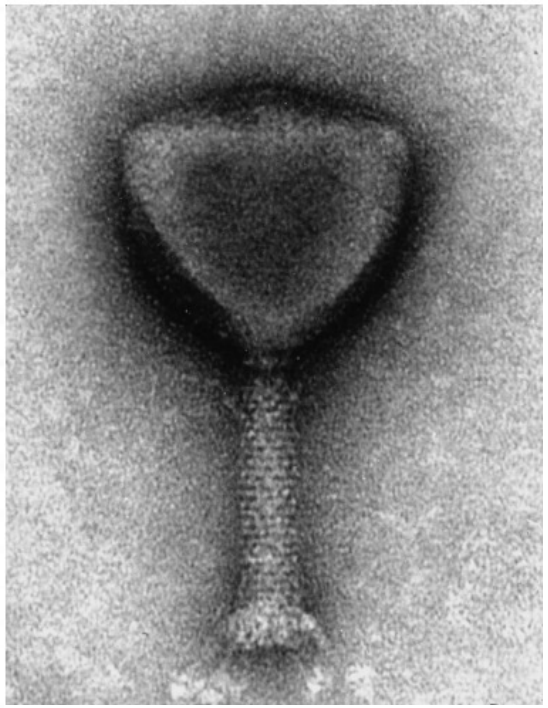
Can we kill *C. sakazakii* with phages?

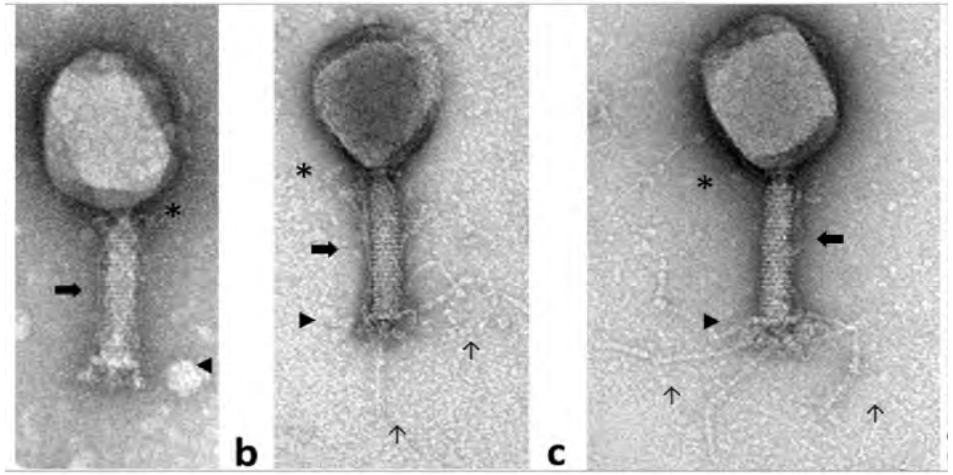
Bacteriophages killing *C. sakazakii*

CR5, myovirus
flagella as receptor



leB, leN, leE are myovirus, stable and efficient between 4 and 37°C, and between pH 6 and 8; 4 log reduction





GAP32, myovirus
very large genome

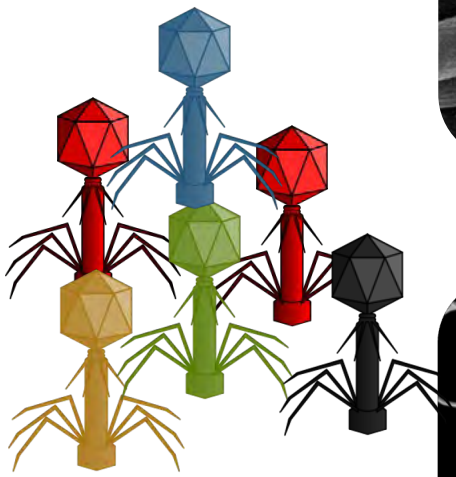
vB_CsaP_Ss1,
podovirus

Endersen et al. 2015, 2017; Lee et al. 2016; Abbasifar et al. 2014

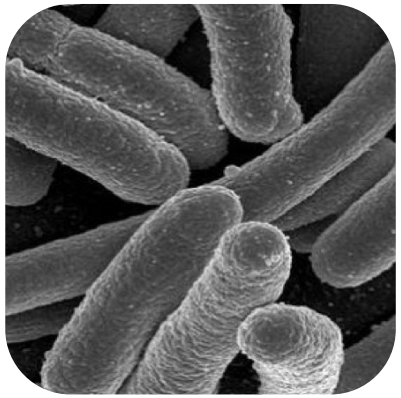
A collection of phages against *Enterobacteriaceae*

TOP SAFE

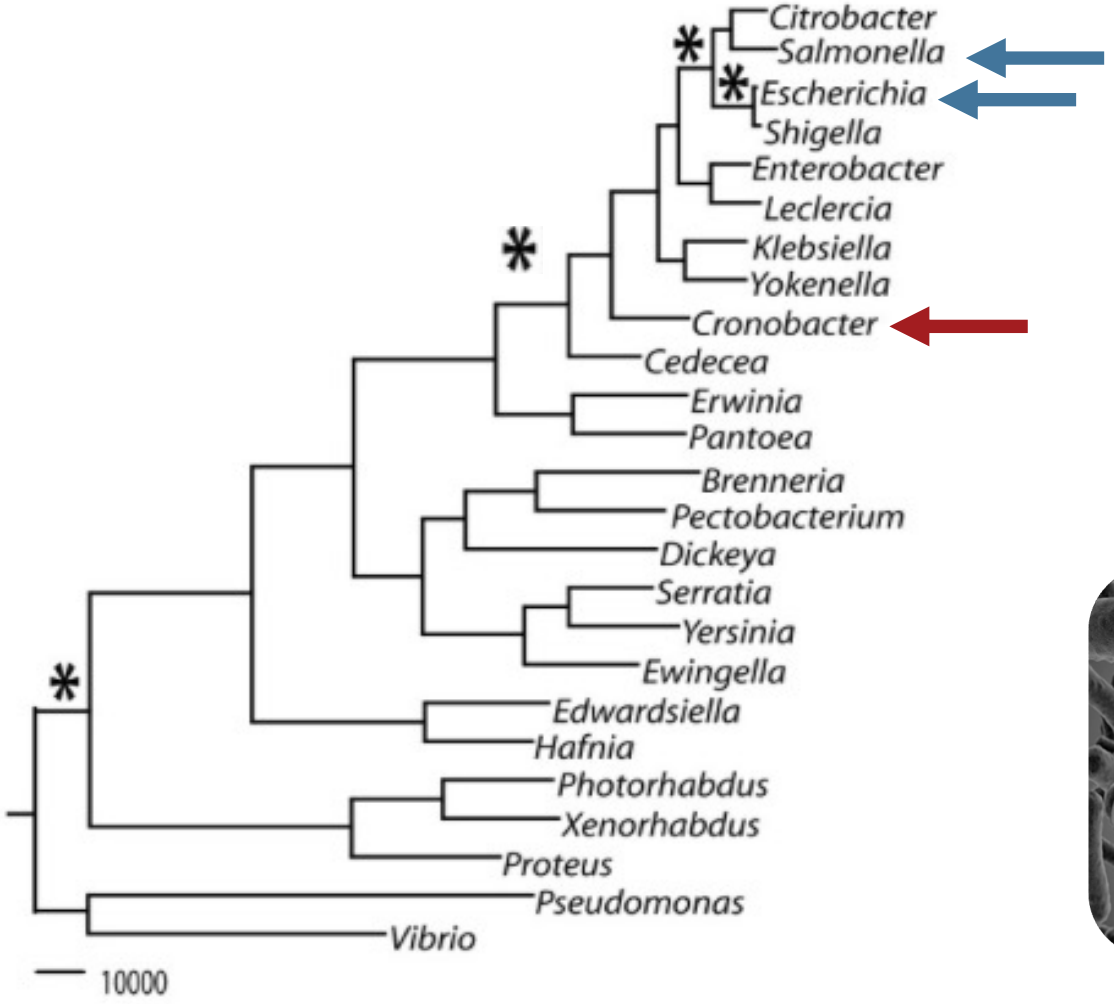
gudp



E. coli

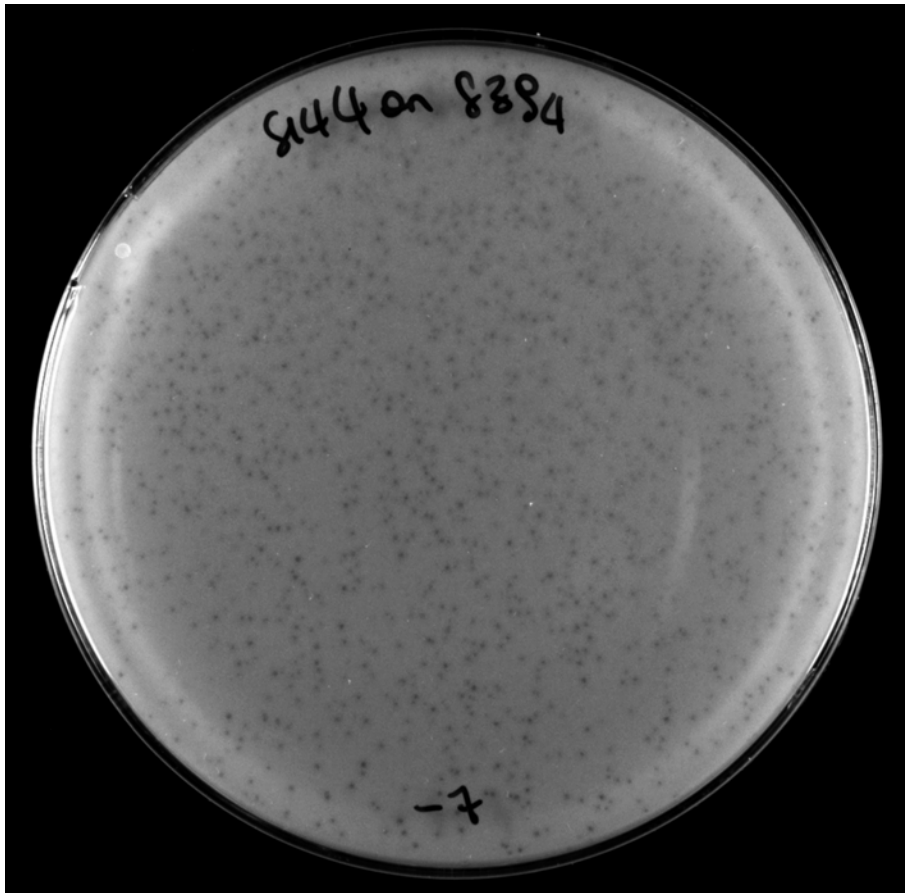


Salmonella

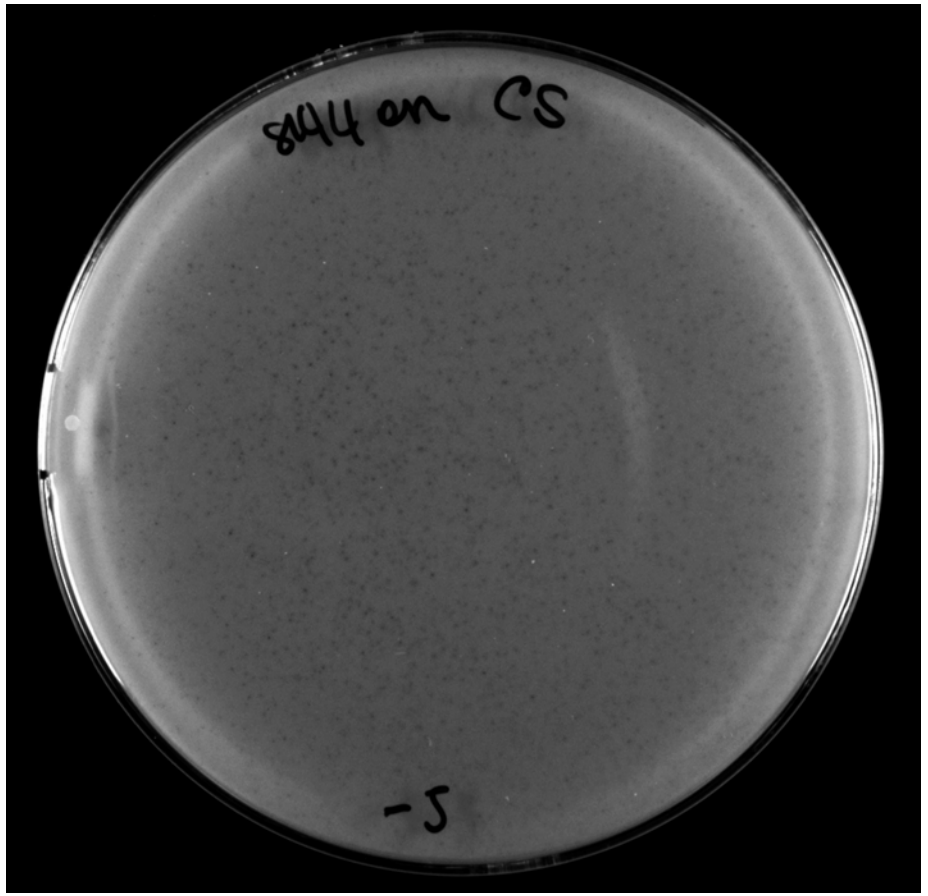


***C. sakazakii*?**

Bacteriophage S144 infects both *Salmonella* and *Cronobacter*



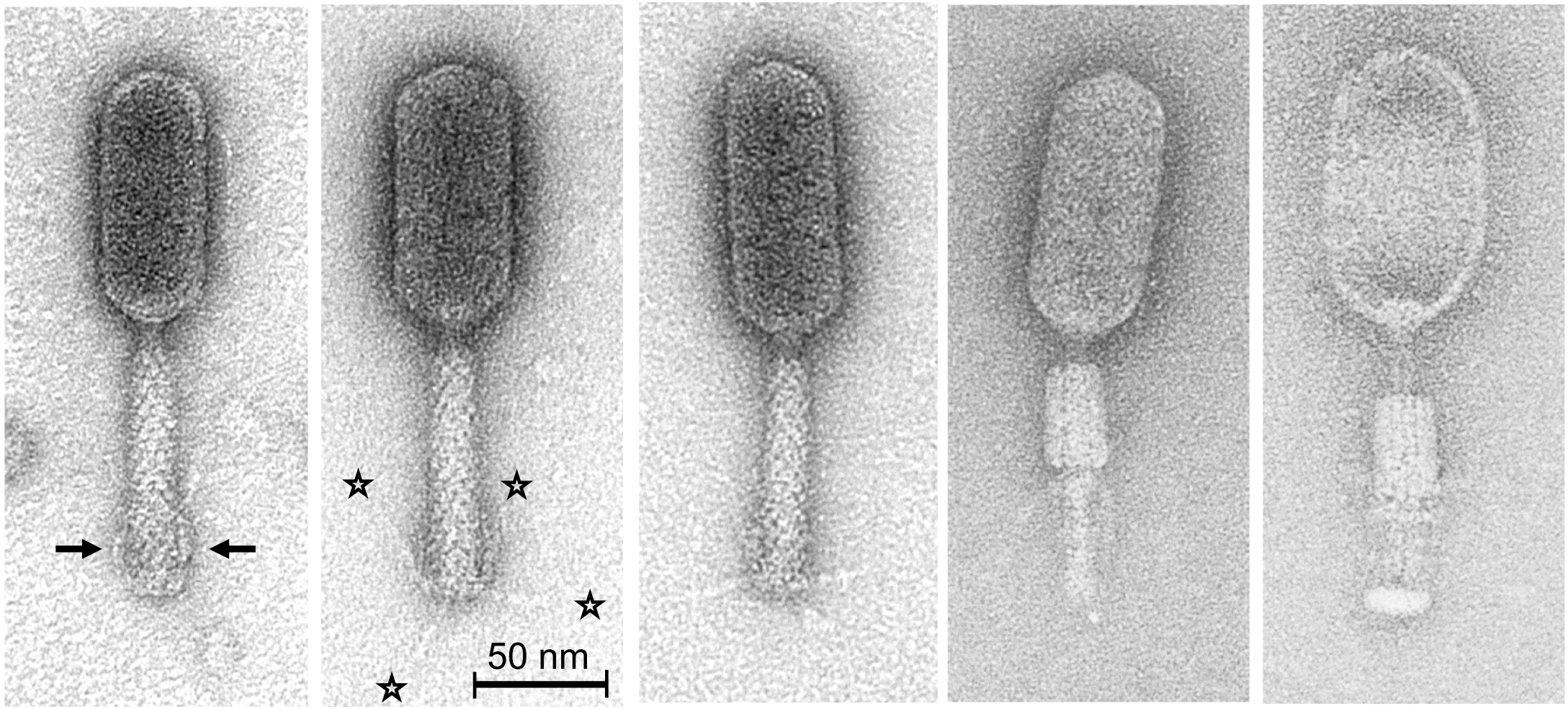
S144 infection of *Salmonella*



S144 infection of *Cronobacter*

Gambino et al., Int. J. Mol. Sci. 2020

S144 is a myovirus, with contractile tail



Gambino et al., Int. J. Mol. Sci. 2020

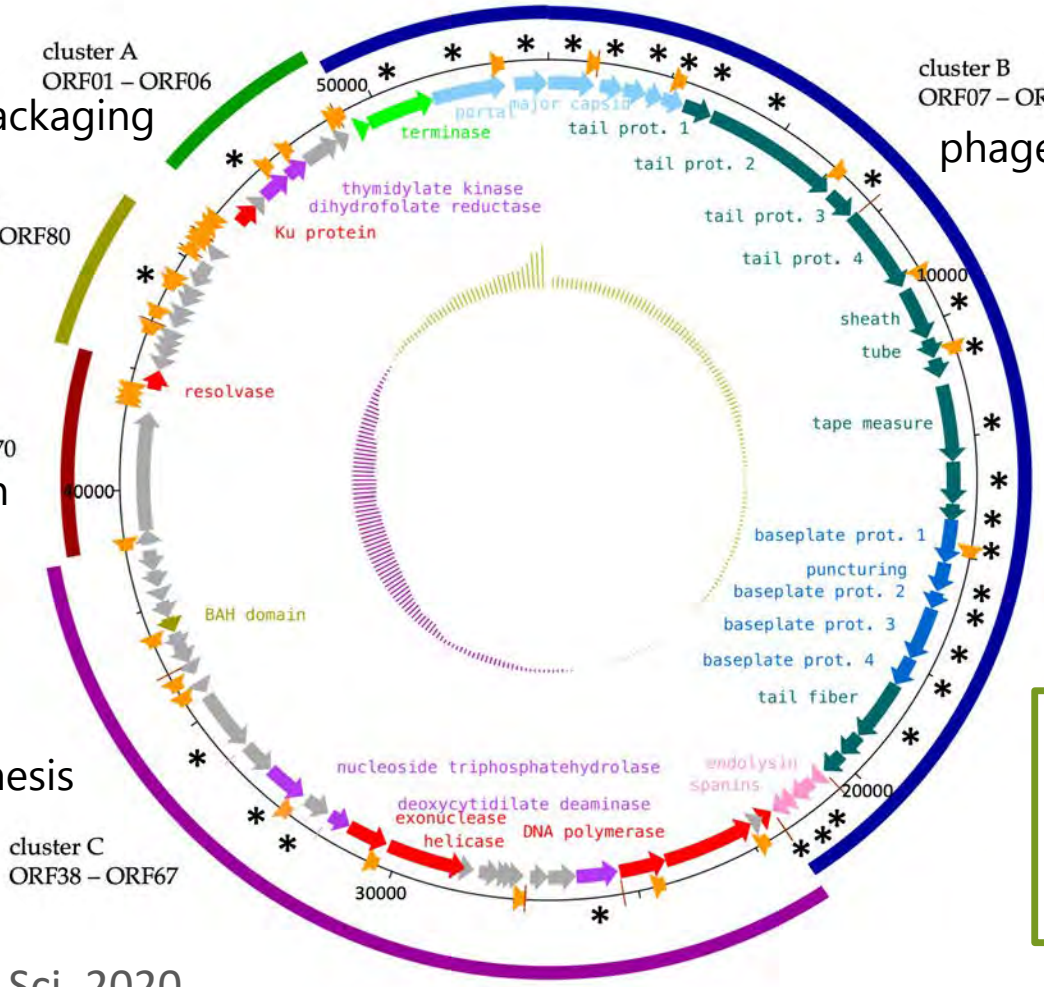
S144 genome is organized in functional modules

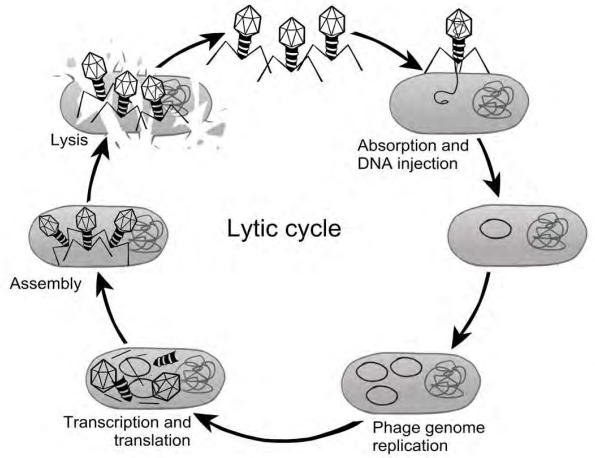
DNA packaging

additional functions

DNA manipulation

nucleotide biosynthesis

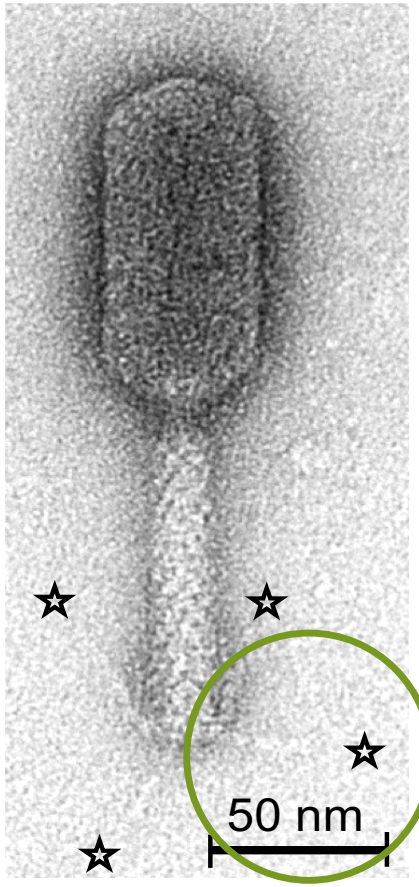




SAFE:
no genes for lysogeny
no genes for antibiotic resistance
no genes for virulence

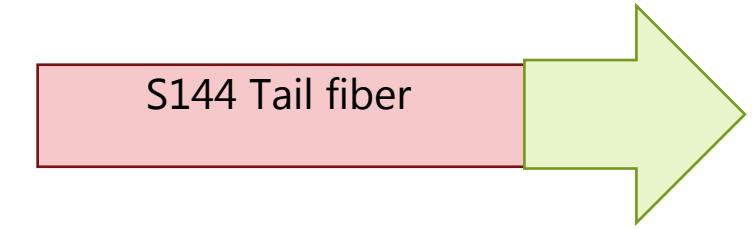
Gambino et al., Int. J. Mol. Sci. 2020

Tip of S144 tail fiber for *C. sakazakii* infection

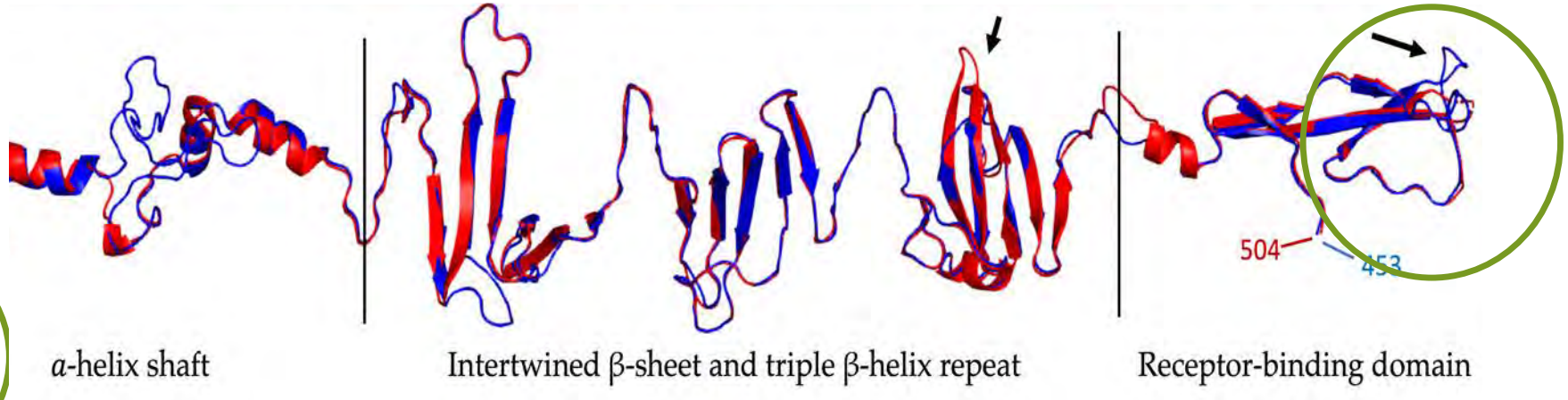


Tail fiber to recognize their host

S144 tail fiber is a mix



Salmonella phages | *Cronobacter* phage



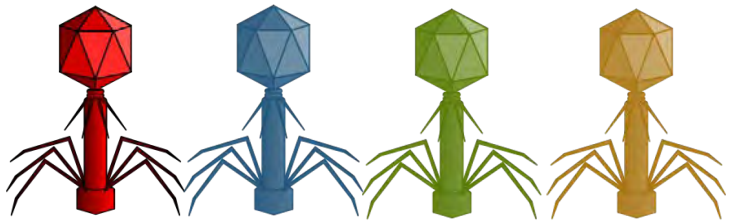
Tail fiber tip similar to other two *Salmonella* phages, SE4 and ZCSE2
→ more *Cronobacter* phages than we think

Gambino et al., Int. J. Mol. Sci. 2020

Next steps

More phages needed

- *Cronobacter* diversity
- phage resistance



Efficacy in real conditions:

- food model in condition relevant for the industrial set-up
- phage production depends on the application: where? when? manufacture, storage, reconstitution?

TOP SAFE

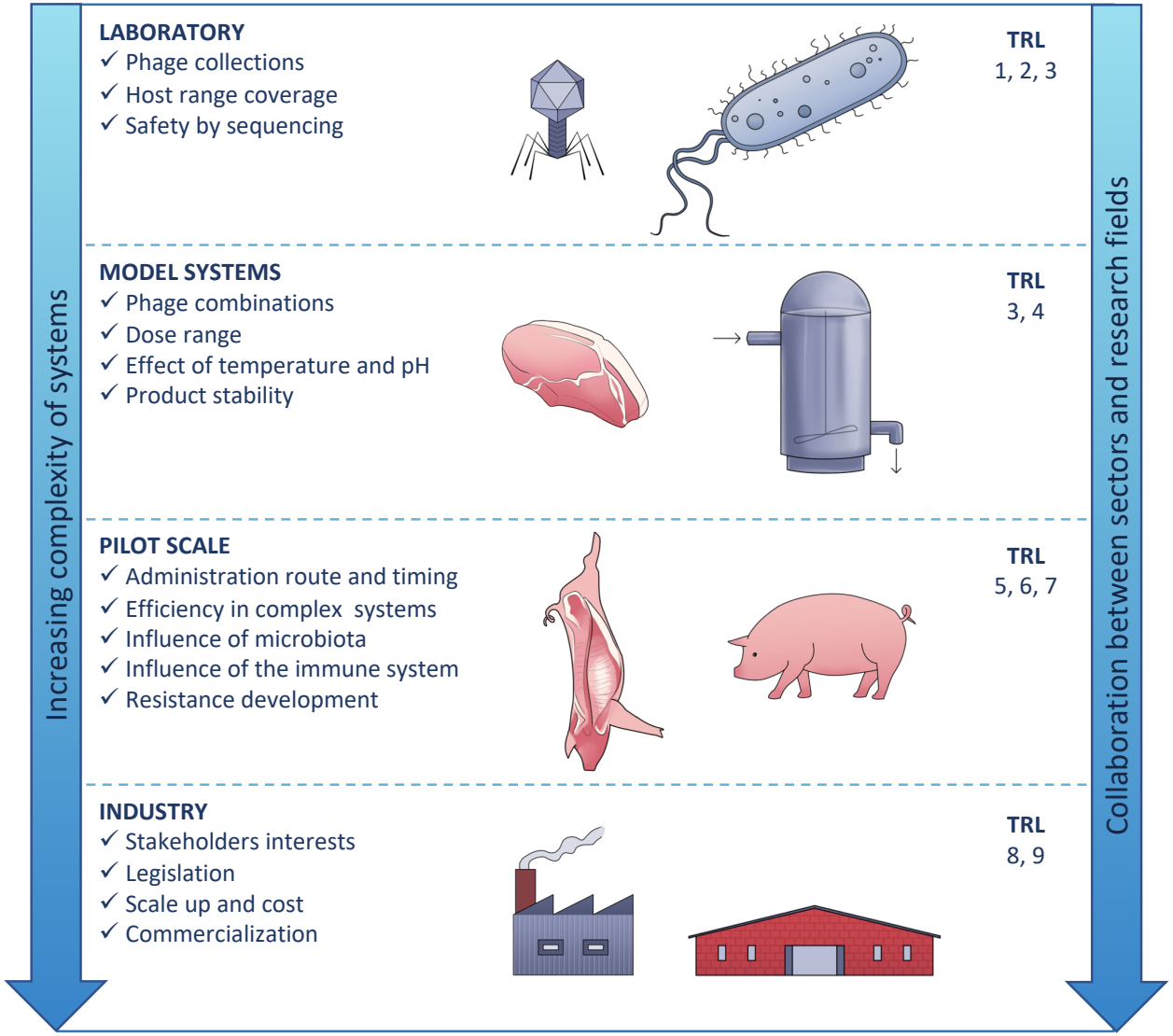
ISI FOOD PROTECTION

Conclusions – can phages help in dairy sector?

Phages can be used to

- target specific difficult pathogens
- target specific species important for the whole community

The approval and use of phages in dairy plants needs involvement of all stakeholders



Gambino and Brøndsted, *Curr Opin Biotech* 2021