The role of phages in the evolution of VTEC and their presence in food and the environment

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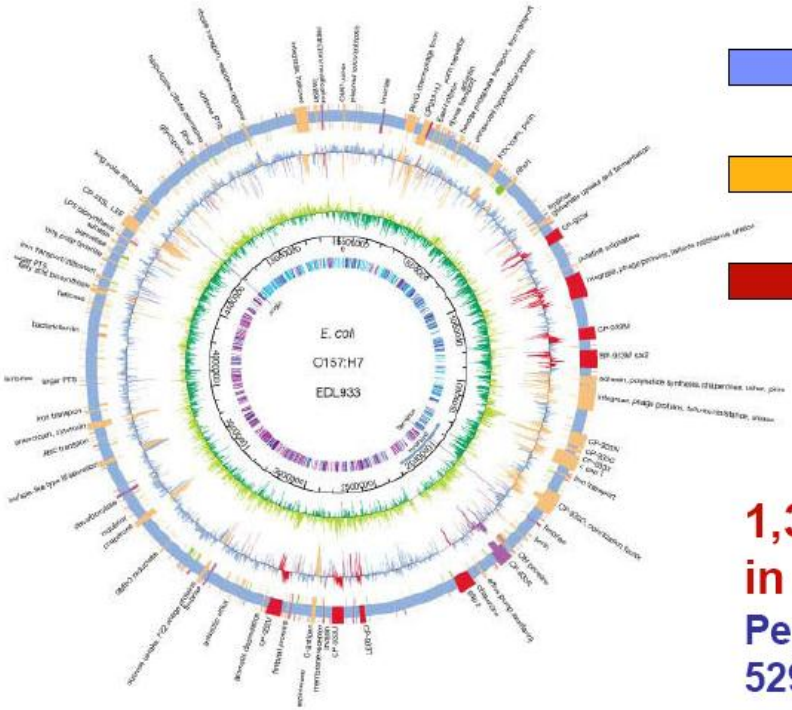
Rome 10th-11rst octubre 2013

Escherichia coli genetic plasticity

E. coli K-12 genome is the “hardware”: **Basic information**
Mobile genetic elements incorporate the “software”. **Armed to be a pathogen**



***E.coli* K12 sequences**

Genomic islands

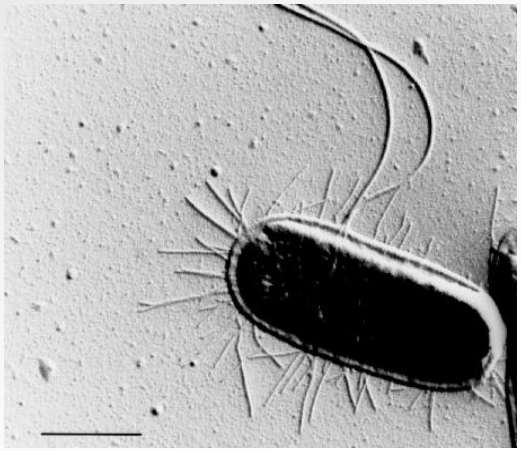
Integrated prophages

1,387 genes absent in E.coli K12
Perna et al. Nature, 409: 529-33, 2001

Not all phages are inducible (cryptic prophages)

Or may be they are and we cannot see it…..

Phages encoding virulent genes in *E. coli*



- **Shiga Toxin**

  Smith *et al.,* J. Gen Microbiol. 1983.

  O'Brien *et al*., Science 1984

- **Genes encoding effector proteins**
  - nleA bacteriophages
  - Cif bacteriophages

  Marchés *et al.,* Molec. Microbiol. 2003

  Loukiadis *et al*., J. Bacteriol. 2008

  Creuzburg et al., 2005

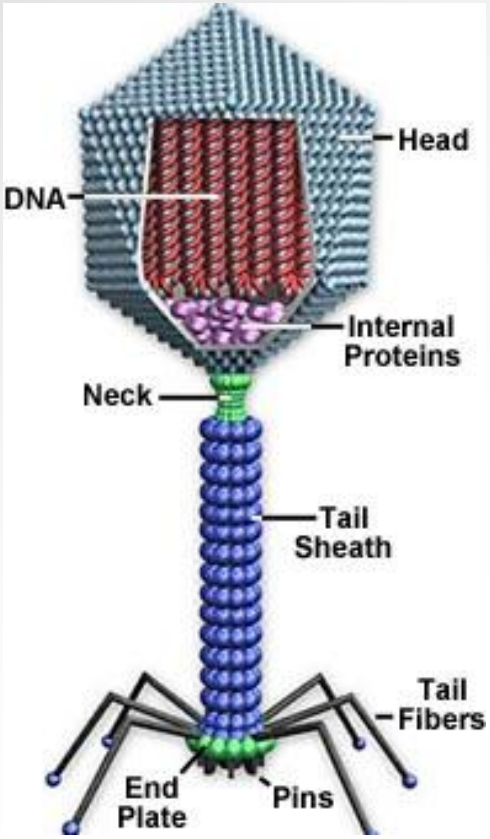
- **CDT (cytolethal distending toxin)**

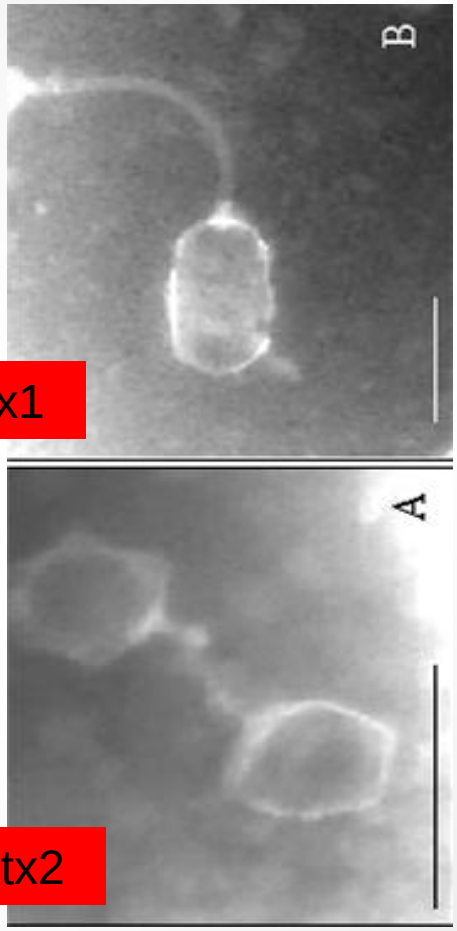
  Janka *et al.,* Inf. Immun. 2003

  Asakura *et al*., PNAS. 2007

  Allué-Guardia et al. Inf. Immun. 2011.J. Virol 2013

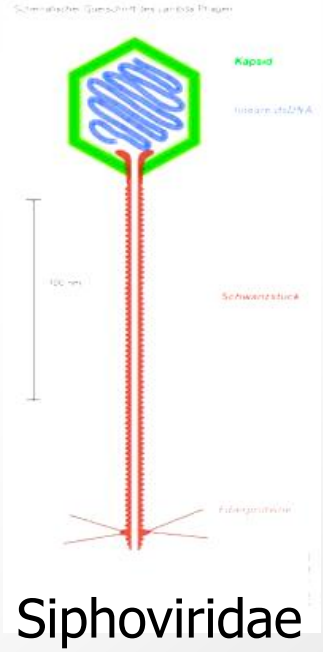
Stx1 and Stx2 phages



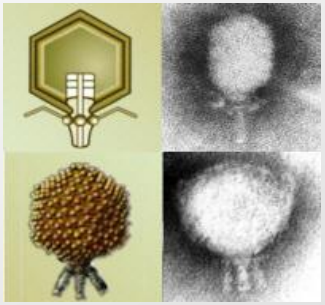


Stx1

Stx2



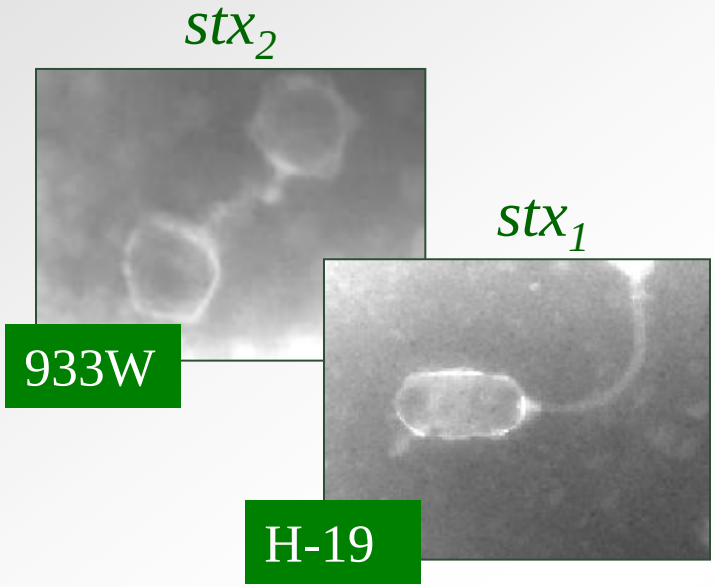
Siphoviridae



Podoviridae

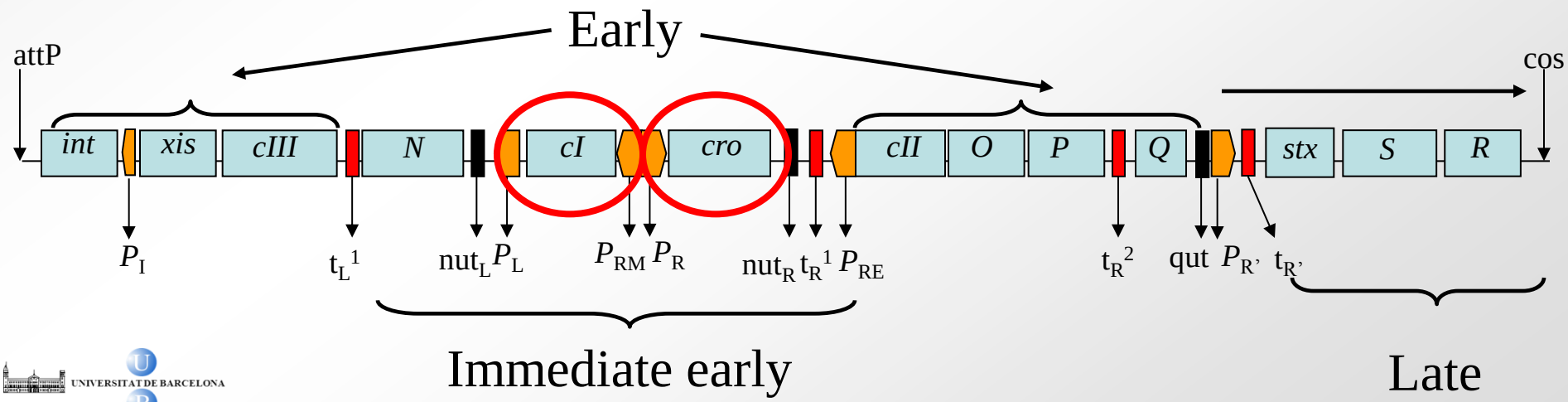
But in general Stx phages are quite diverse (morphology, host range, genetics…)

Shiga-like toxins: Stx_1, Stx_2, variants of Stx_2

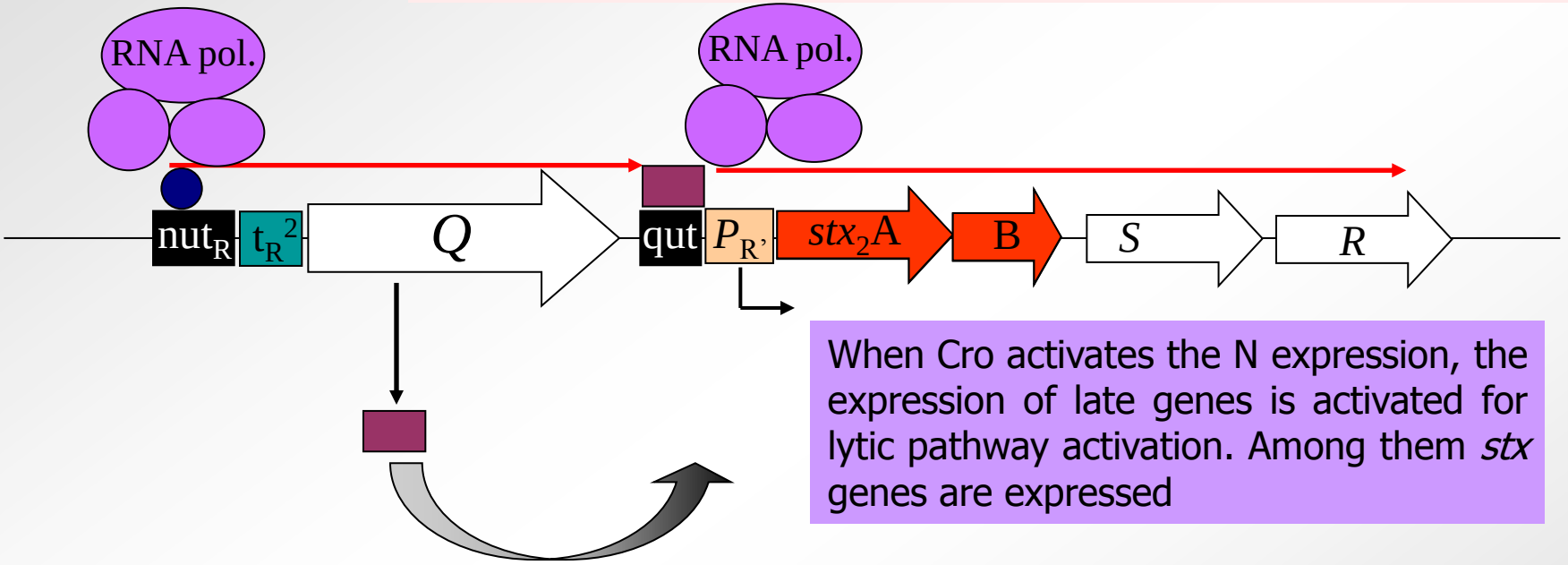


stx genes are encoded in the genome of temperate bacteriophages

stx2e, stx2d, stx2c, stx2g



Induction of Stx phages



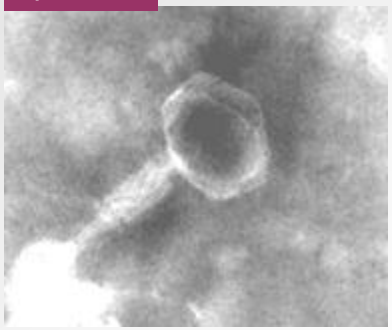
When Cro activates the N expression, the expression of late genes is activated for lytic pathway activation. Among them *stx* genes are expressed

♣ Phage lytic cycle induction by activation of bacterial **SOS response** (recA dependent or not) can be activated by:

- U.V. radiation.
- Certain antibiotics (Mitomycin C, fluoroquinolones, beta-lactamics…)
- EDTA and other chelating agents
- Hydrogen peroxide
- Iron depletion: only for Stx1 without phage lysis
- Food treatments !!! (heat, radiation in low doses, antibiotics as prophylactics, HHP…)

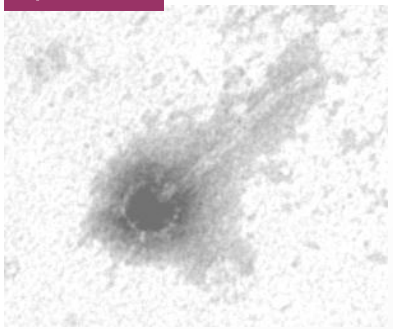
Diversity: Morphology

ϕ1a

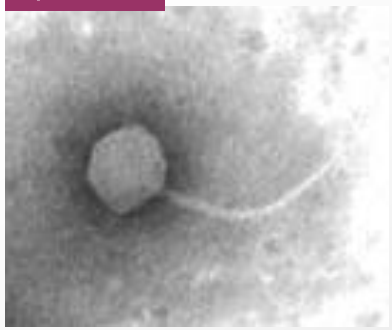


ϕ4

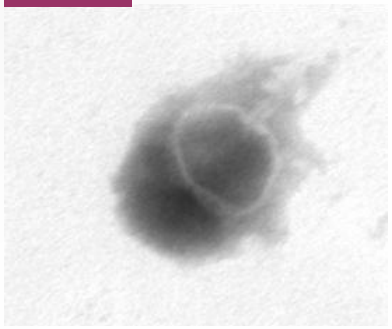
ϕ13a



ϕ16a



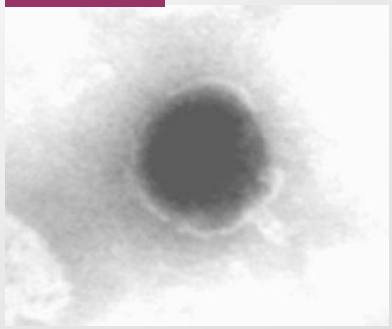
ϕ1b



ϕ12

ϕ13b

ϕ16b

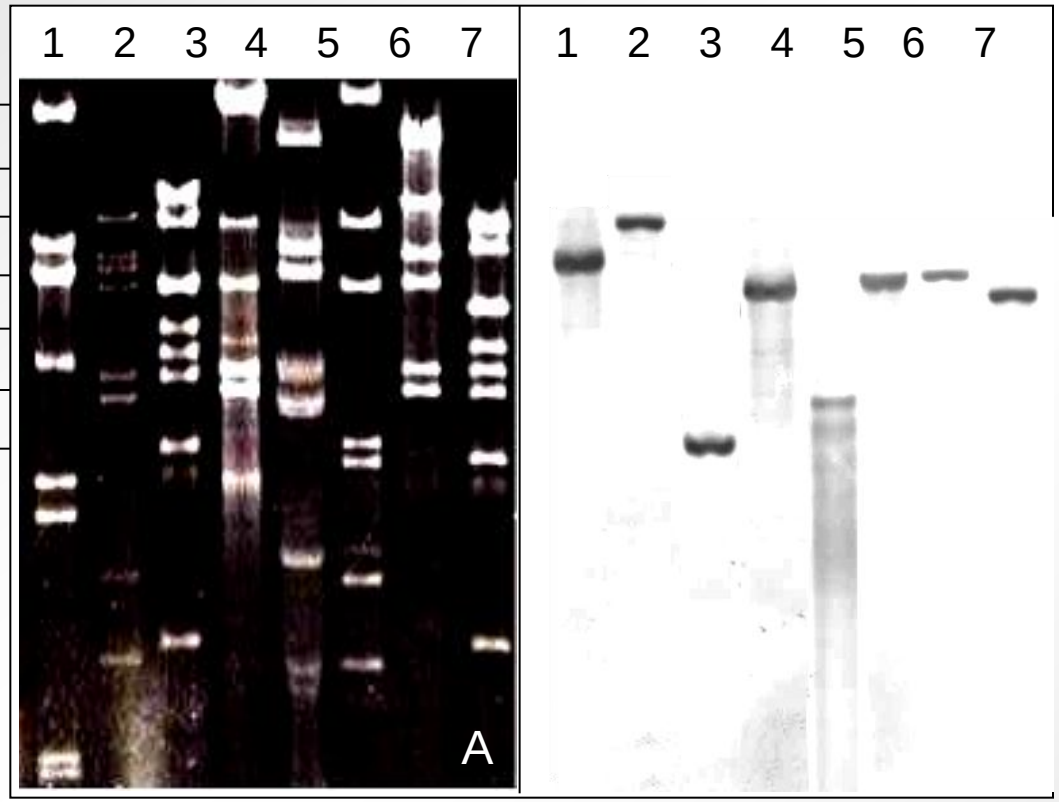


Diversity: Host range

Host bacteria	Strain	Phages									
		ϕ1a	ϕ1b	ϕ3	ϕ4	ϕ5	ϕ6	ϕ12	ϕ13a	ϕ13b	ϕ16b
Shigella dysenteriae	500	+	+	+	+	+	+	-	+	+	+
Shigella boydii	316	-	+	-	+	-	-	-	-	-	-
Shigella boydii	351	-	+	+	+	-	-	-	-	-	-
Shigella sonnei	866	+	+	+	+	+	+	-	+	+	+
Shigella sonnei	635	+	+	+	+	+	+	-	+	+	-
Shigella flexnery	805	-	+	-	+	-	-	-	-	-	-
Shigella flexnery	668	+	+	+	+	+	+	+	+	+	+
E. coli O111	209	-	-	-	+	-	-	-	-	-	-
E. coli O26	216	-	-	-	+	-	-	-	-	-	-
E. coli O26	224	-	-	+	+	+	+	-	-	+	+
E. coli O157:H7	ATCC43888	-	-	-	+	-	-	+	-	-	-
E. coli C600	-	+	+	+	+	+	+	+	+	+	+
E. coli DH5 α	-	+	+	+	+	+	+	+	+	+	+
E. coli WG5	-	+	+	+	+	+	+	+	+	+	+

Shigella dysenteriae	90 %	*Shigella boydii*	85 %
Shigella sonnei	25 %	*Shigella flexnery*	60 %
E. coli O111	10 %	*E.coli* O26	35 %
E. coli O157:H7	20 %	*E. coli* C	100 %

Variability among phages



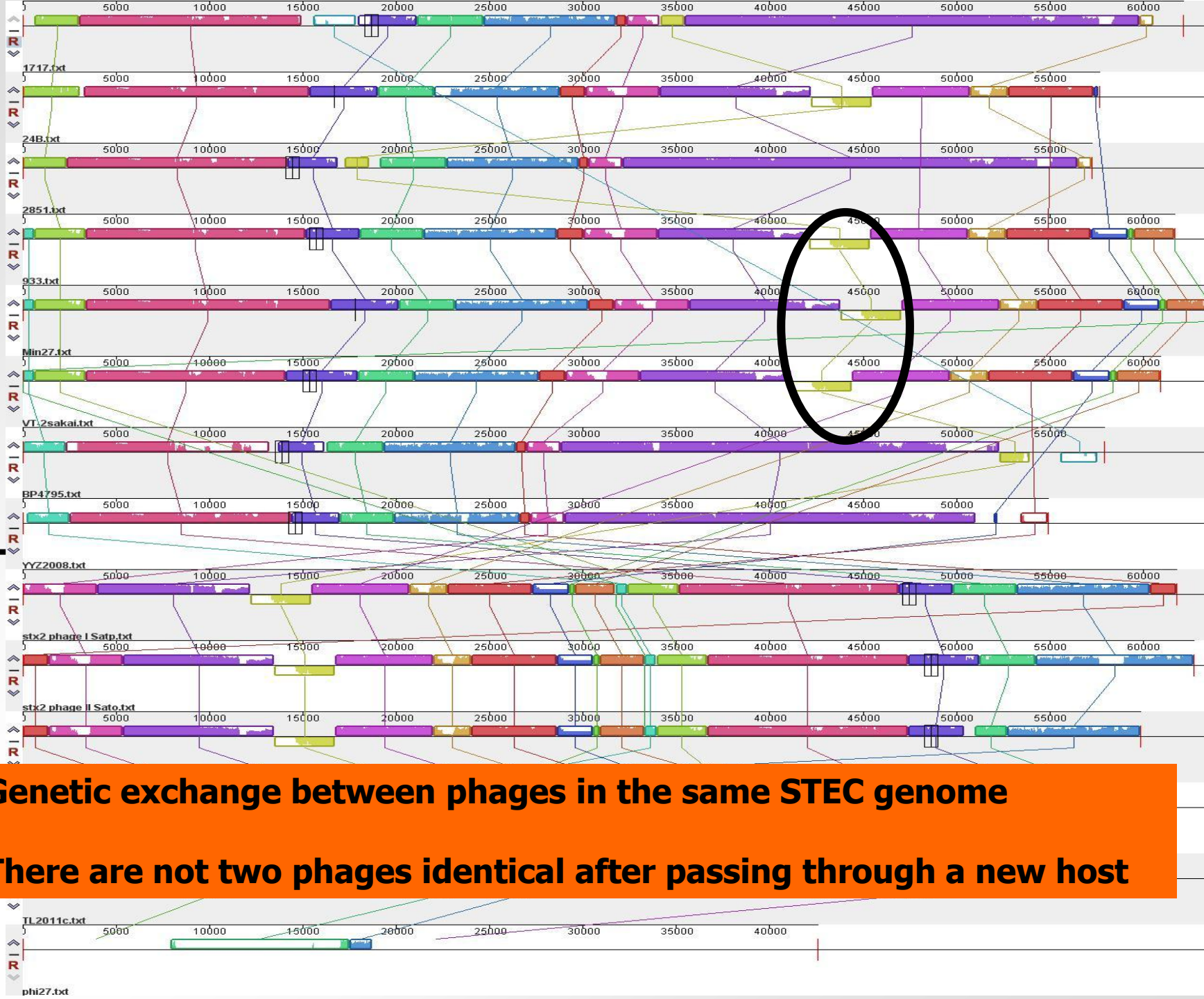
Southern blot stx2A-DIG probe

phage DNA of 7 different Stx phages isolated from the environment

Muniesa *et al*., Microbiology UK. 2004

Stx2 phages

Stx1 phages



Genetic exchange between phages in the same STEC genome

There are not two phages identical after passing through a new host

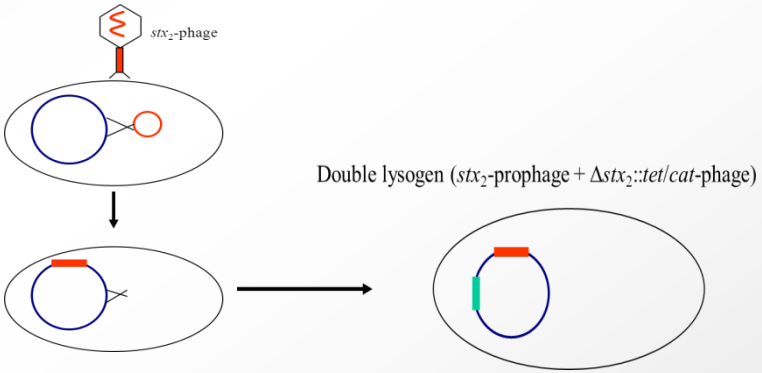
Stx phages cause variability in STEC

- Stx phages are involved in the horizontal transmission of the gene: TRANSDUCTION
  - in the lab (Schmidt *et al.*, 1999)
  - in the intestinal tract (Toth *et al.*, 2003; Cornick *et al.*, 2006)
  - in biofilm (Solheim *et al.*, AEM 2013)
  - In food and water

Emergence of new virulent strains

Double lysogens

- Some STEC strains isolated from food, environment and clinic carry more than one inducible Stx phage in their genome (65 %)
- In the double lysogeny of Stx phages does not apply the phage immunity described for phage lambda



-Have a direct implication in Stx production

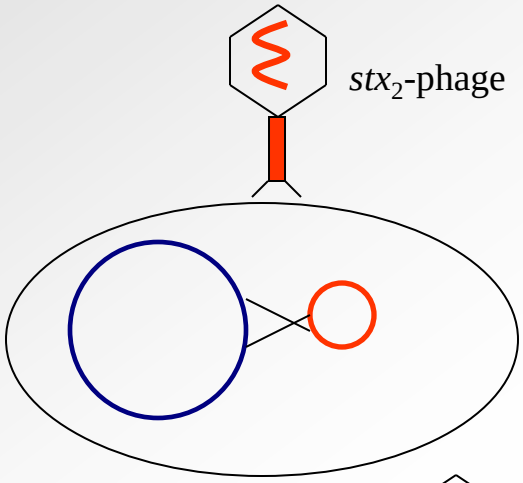
-Increase **genome** variability by allowing recombination between both phages.

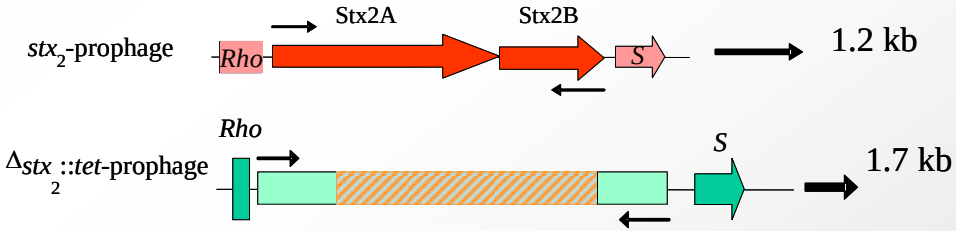
Serra-Moreno et al., J. Bacteriol, 2008

Stx phages search for insertion in E. coli chromosome

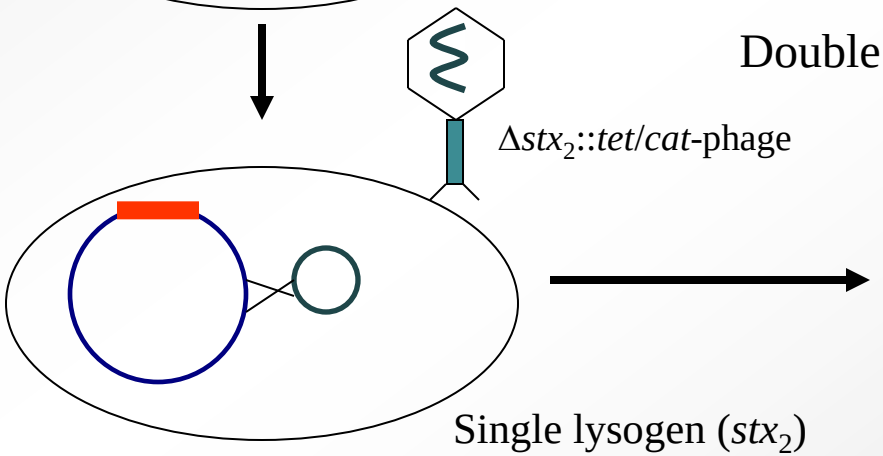
Double and even triple lysogens of the Same Stx phage could be generated

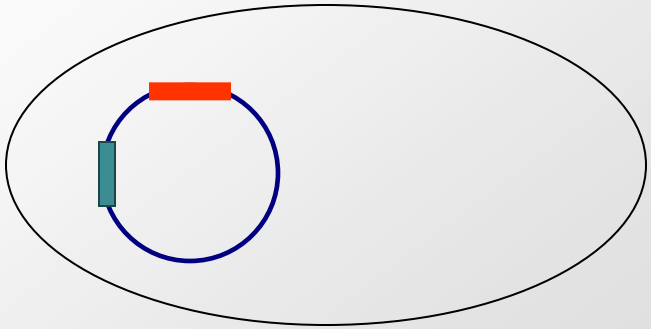
They have a preferred insertion site. If it is not available they search for a secondary site for insertion.





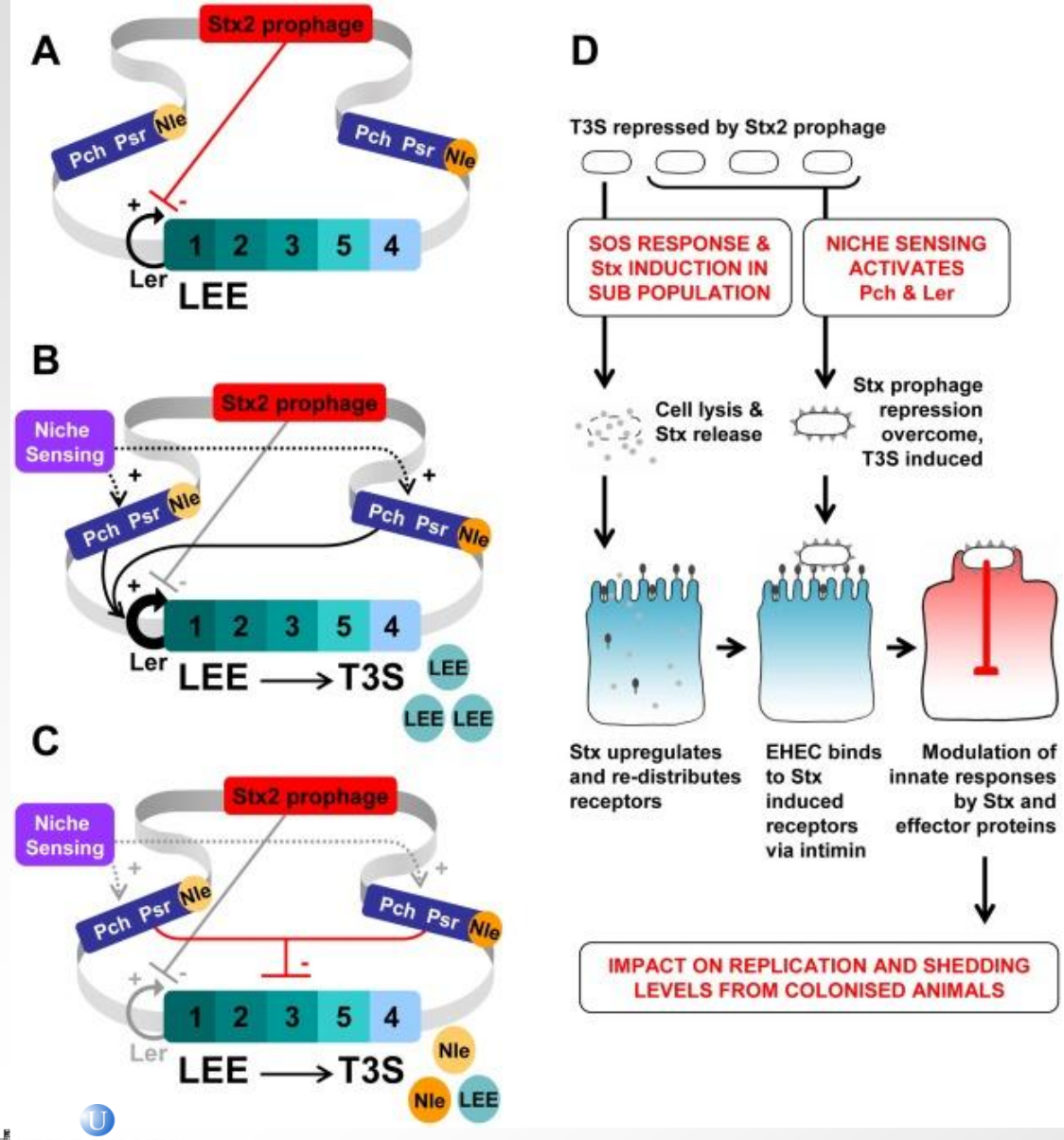
Double lysogen (stx_2-prophage + Δstx_2::*tet*/*cat*-phage)





Single lysogen (stx_2)

Stx2 phages down regulate LEE expression

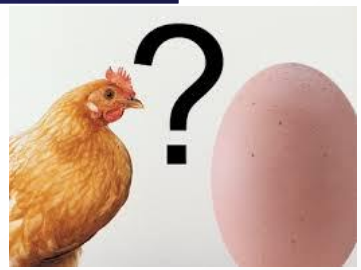


Deletion of Stx2 phages from EHEC strains increased the level of T3SS in LEE whereas lysogeny by Stx2 phages decreased T3SS.

Xu et al., PLoS Pathog. 2012

What is the origin of Stx phages?

Are they induced from VTEC?
Are free-Stx phages moving around and generate new VTEC?



Food

Feces
- humans
- animals

Environment
- Water
- Soil
- Vegetables

Stx phages in the environment

Stx2 phages occurring in sewage were detected by qPCR.

- Abundant in fecal polluted water (human and animal) in Barcelona
  - More than 1000 per ml of sewage
- Present in different geographical areas
  - Austria, France, **Germany**, Ireland, UK, New Zealand, Spain, Sweden or South Africa
- Persistent
  - Chlorination, natural inactivation, thermal treatment, UV light

Stx phages from these samples can propagate in *E. coli* , showing that some of the phages detected are infectious.

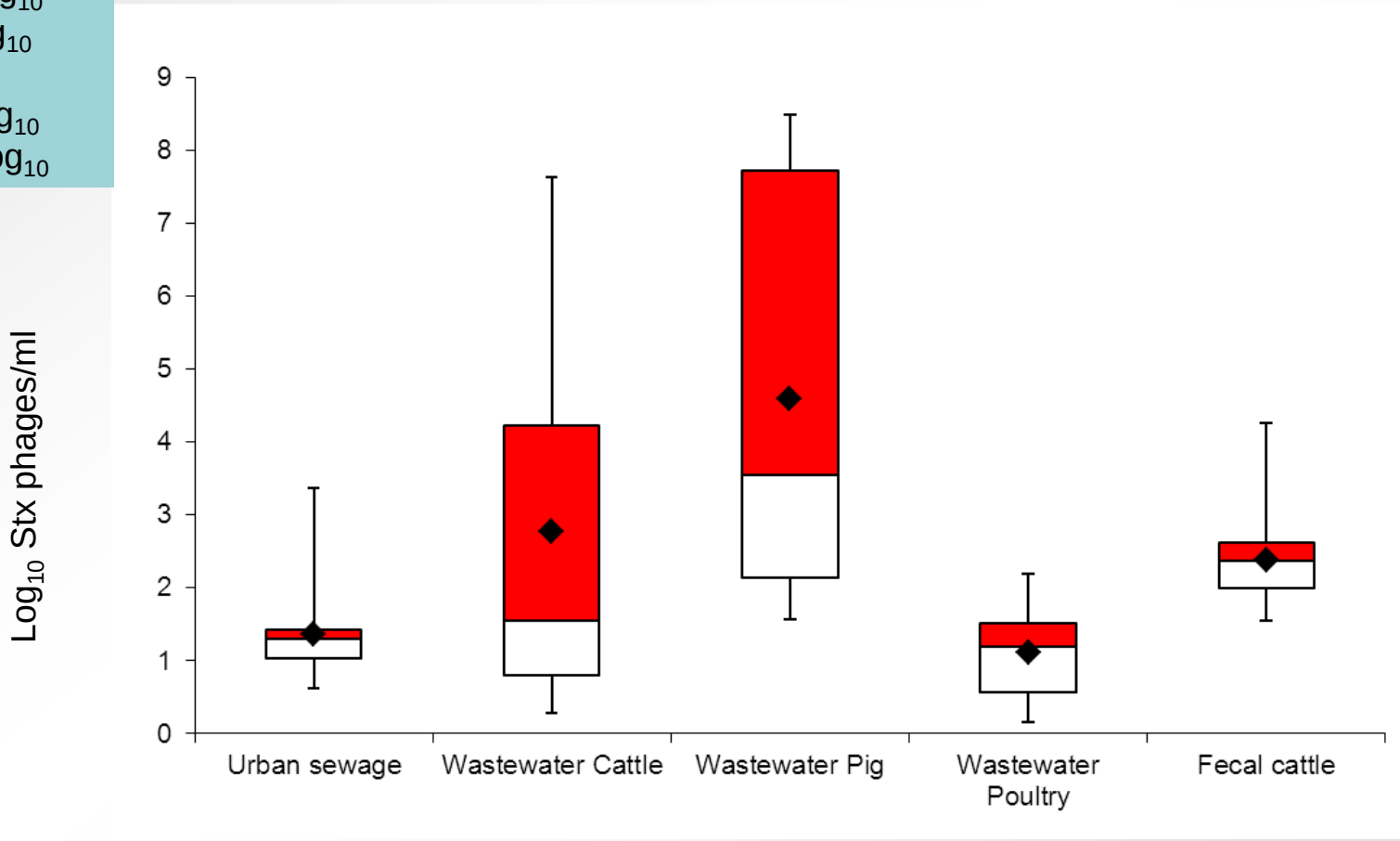
Infectivity means they are potentially able to transduce *stx*.

Muniesa & Jofre, AEM 1998; Muniesa *et al.*, AEM 1999; Muniesa & Jofre, FEMS Microbiol. Lett. 2000; Imamovic et al., AEM 2010

Dumke et al., Lett Appl. Microbiol. 2006; Tanji et al., Water Sci Technol. 2002; Rooks et al., Environ Microbiol. 2010

Faecally polluted environments

Human: 1.4 log_{10}
Cattle: 2,8 log_{10}
Pig: 4,6 log_{10}
Poultry: 1.1 log_{10}
Slurries: 2,3 log_{10}



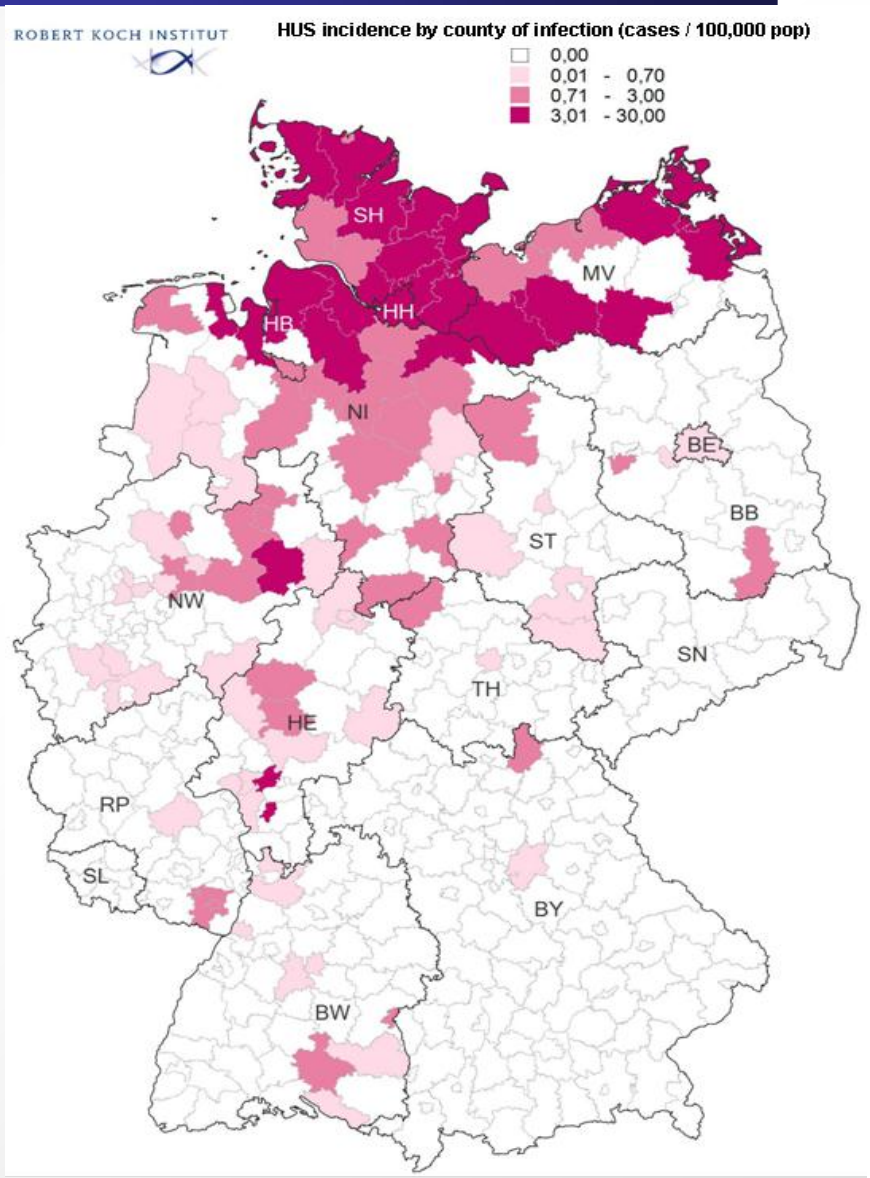
Imamovic et al., AEM 2010

O104:H4 outbreak in Germany

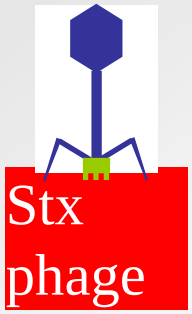
Shiga toxin-producing *E. coli* in Germany. May 2011

2nd June 2011 more than 3255 confirmed cases, 800 with HUS high number of deceased (33).

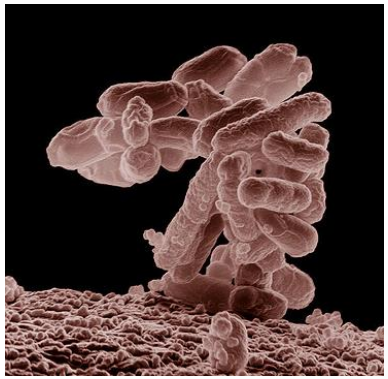
The 3rd most relevant outbreak of *E. coli* in recent history and the greatest in Germany



Emergence of a new strain



Unknown origin



Enteroaggregative *E. coli* (O104:H4), Stx -, with aggregation factors

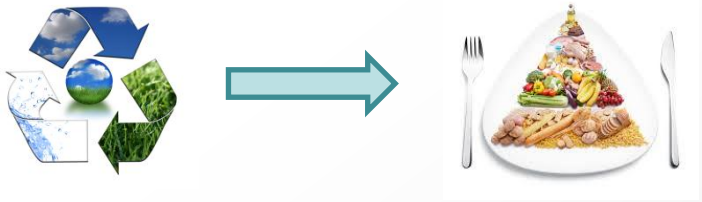
Enteroaggregative E. coli Stx-producer
- Serotype O104:H4
- ***stx*2a+**, *eae*-, *hly*-
- **aggregation factors+**

What is the origin of O104:H4 ?

Epidemiological data showed vegetables as the source of the strain.

However, O104:H4 was NOT isolated from more than 800 samples of sprouts analyzed.

All indicated that **Fenugreek sprouts** from **Egypt,** with a clear coincidence in France and Germany, could be the origin of the causative agent of the German outbreak.



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Food

Feces
- humans
- animals

Environment
- Water
- Soil
- Vegetables

Stx phages in food

Food

Microbiological criteria EU regulation

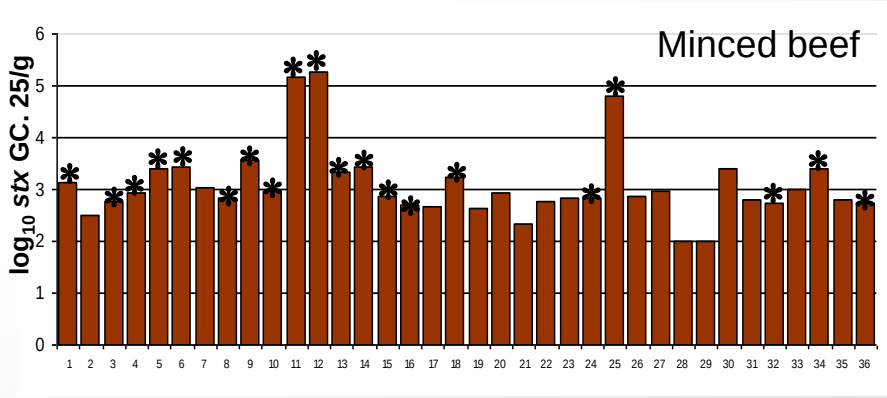
10^2-10^5 Stx phages / 25 g minced beef. 100% of the samples
10^2-10^3 Stx phages / 25 g fresh salad. 69 % of the samples

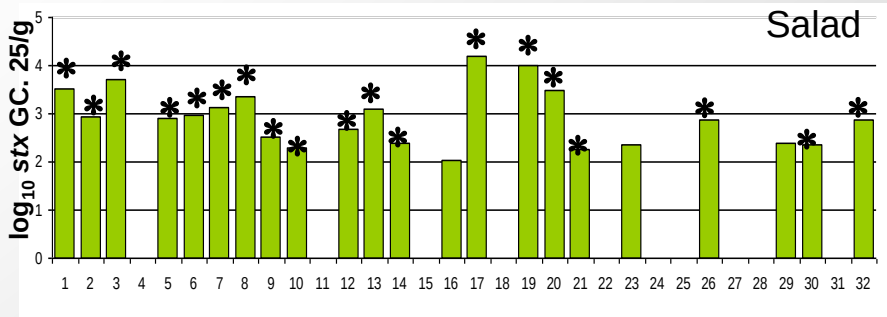
Bar: qPCR
*: + end point PCR

At least 50 % of the Stx phages in these samples were infectious

Able to propagate in host strains

Barcelona area has a low incidence of VTEC infections

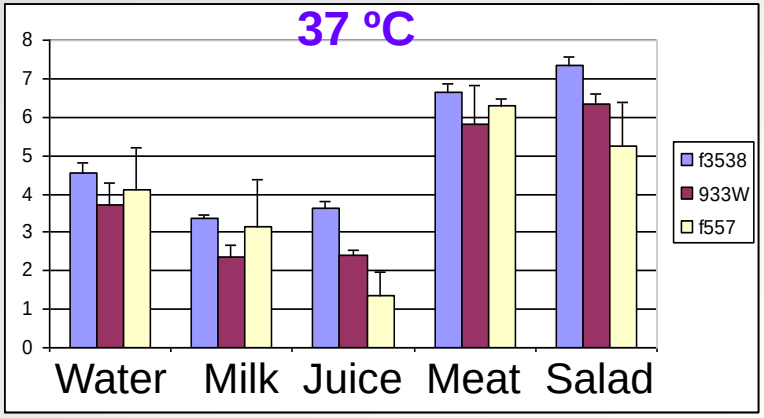


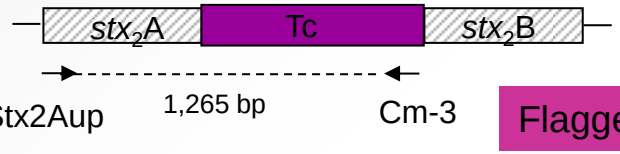


Sample nº

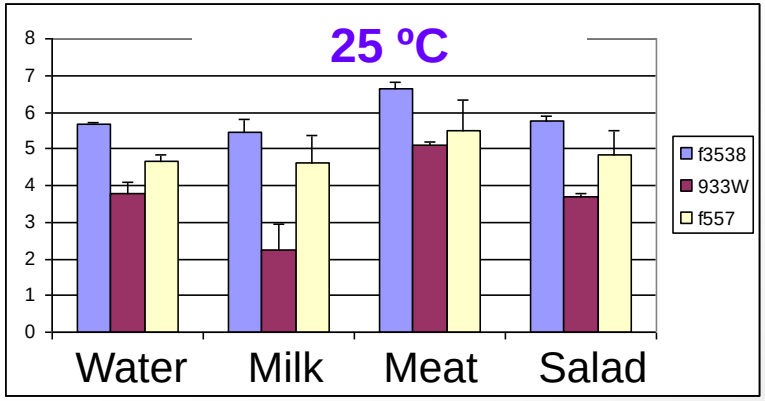
Imamovic & Muniesa, AEM. 2011

Stx phage transduction to *E. coli* can take place in food





Flagged phages



Imamovic et al., AEM. 2009

- To various *E. coli* strains (including O157:H7)
- In liquid or solid matrices
- A minimal number of host and phages are required
- Without inducing agent

➢ Not at 4ºC!
➢ Not at low pH (below 4)

Transduction in milk at different temperature profiles and cell concentrations

- ➢ Transduction was not affected by initial or final concentration of donor or recipient strains.
- ➢ occurs when the cells are metabolically active
- ➢ it does not take place at low temperatures.

Picozzi et al., Int J Food Microbiol. 2012

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Are free-Stx phages moving around?

Food

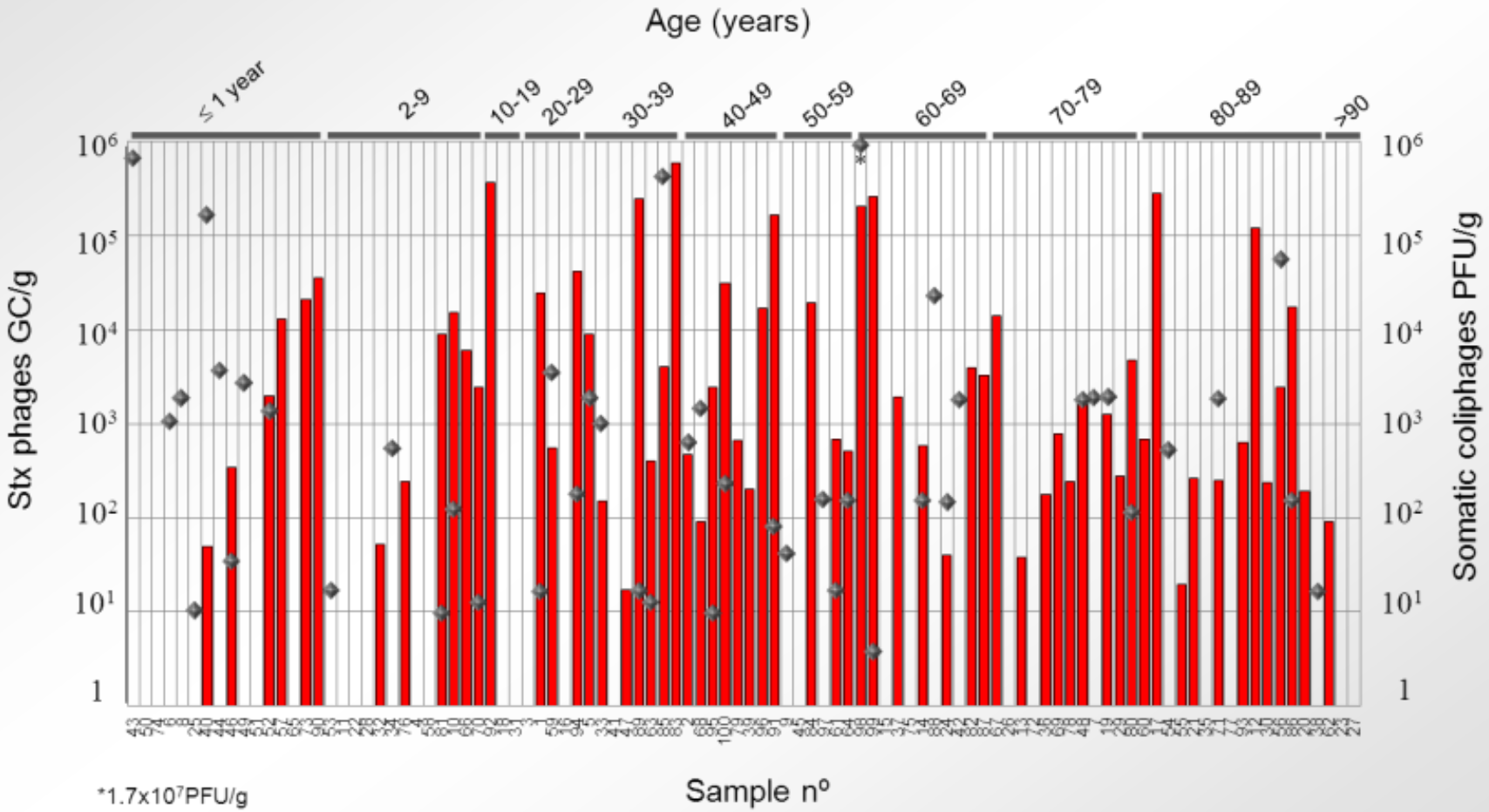
Feces
- humans
- animals

Environment
- Water
- Soil
- Vegetables

Stx phages in human feces (healthy individuals)

2.6 x10^4 Stx phages/g
62 % positive samples

Infectious Stx phages in 95% of positive samples



Methods for phage DNA extraction → Filtration to recover phages → Proteinase to break the phage capsid → Phage DNA purification

DNAse

Methods for bacterial DNA extraction → Propagation of bacteria in selective medium → Lysis to break the bacterial cell → Bacterial DNA purification

Only bacterial??

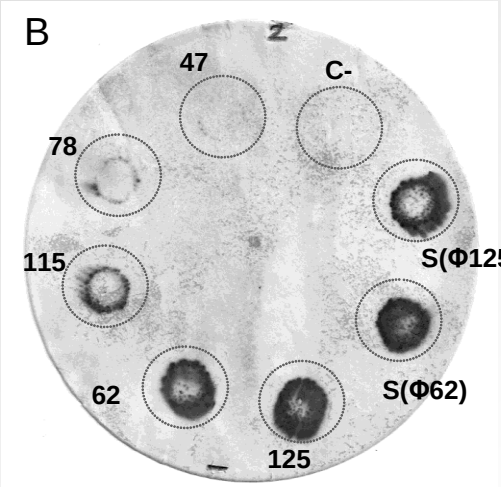
You can also propagate lytic phages. **Lysis of the target bacteria**

You can also break phage capsids

When looking at *stx* genes in stools (or in other samples) by PCR are we always detecting STEC or could be detecting Stx phages?

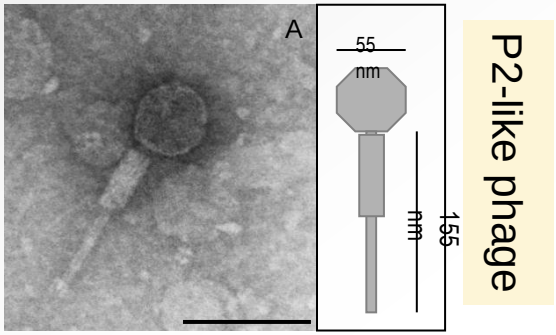
DNA extraction methods can include both!!!

Other phages in STEC. Cdt phages

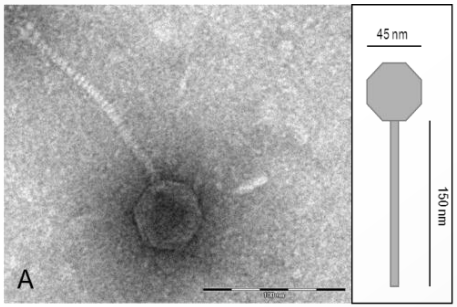


Phages carrying diverse *cdt* variants

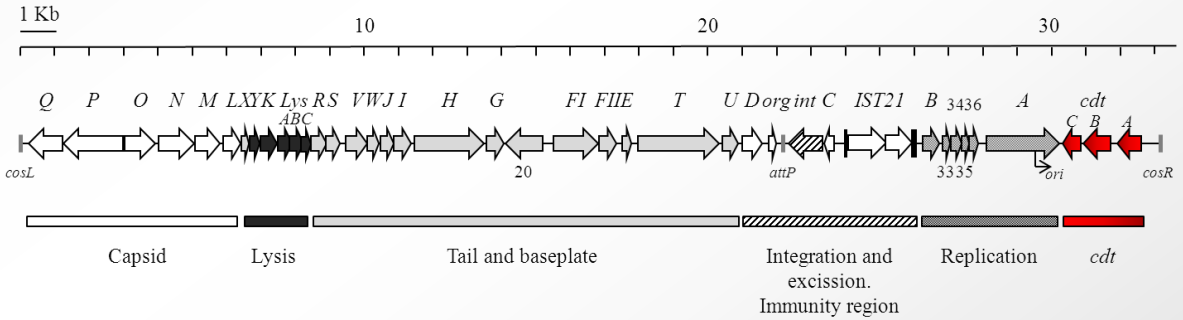
Cytolethal distending toxin, causes distension in the cells and blocks mitosis



P2-like phage



Lambda phage



- Variability among phages described
- Transduce *cdt* to *E. coli* and to *Shigella*
- Present in wastewater (10^3 GC/ml)
- High persistence to disinfection

Asakura et al., PNAS 2007; Toth et al., Infect Immun 2009;
Allué-Guardia *et al*., InfectImmun 2011; J. Virol 2013; Svab et al. AEM, 2013

Phages encoding genes of effector proteins

Cif bacteriophages

Loukadis et al. J. Bacteriol. 2008

Cif: T3SS mediated effector protein that cause cytostatic effect

Is encoded in a lambdoid prophage present in EPEC and EHEC

Other genes encoding effector proteins (nle) are found in the genome of these phages

Hot spot for insertion of bacterial genes

▪nleA bacteriophages

nleA gene and also linked to other effector genes

Apparently these phages were not inducible

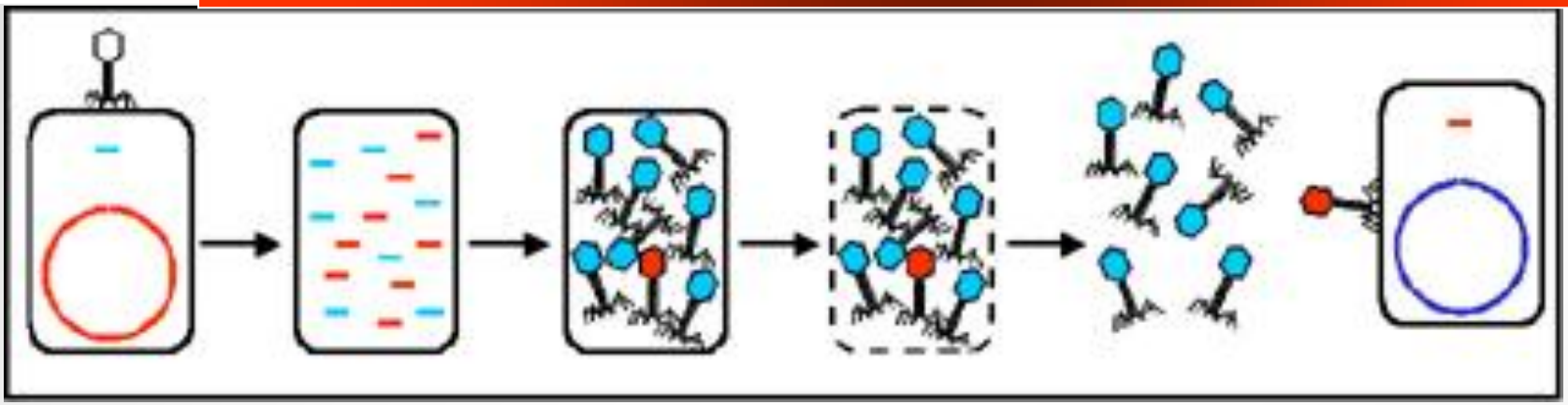
Prophages may have integrated type III effector genes during recombination events in the lytic life cycle

Creuzburg et al. AEM 2011

The majority of functional effector genes are encoded by 9 exchangeable effector loci that lie within lambdoid prophages. Thus, T3S in *E. coli* is linked to a vast phage "metagenome". **The major function of lambdoid prophages in EHEC is to carry T3S effectors.**

Tobe et al., PNAS. 2006

Phages can mobilize **ALL GENES** through **generalized transduction!**



- Phage-derived generalized transducing particles are a phage capsid with bacterial DNA inside.

  1.-Equal high persistence in the environment
  2.-They can infect new hosts and transduce these genes
  3.-They will not release as new phages again

- Some processes increase the amount of transducing particles and hence the frequency of transduction and emergence or new strains.

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Thanks!

Gracias!

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