

The multiple facets of bacteriophages and their role in the diversity and virulence of *C. difficile*

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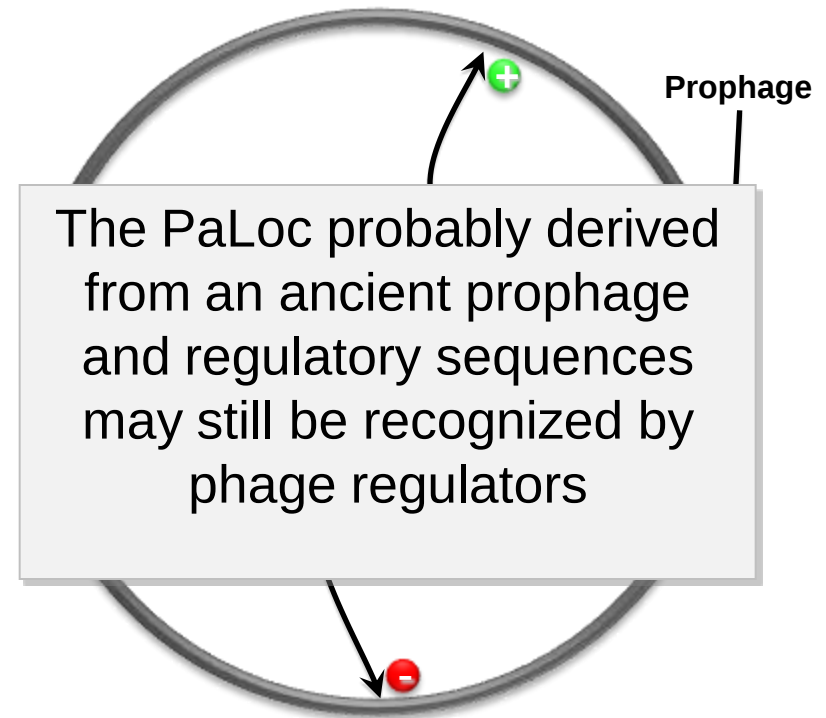
The complexity of *C. difficile*

- Toxins A and B are the main and essential virulence factors in *C. difficile* (Kuehne, Nature 2010)
- *C. difficile* infections are complex and multifactorial
- Several genotypes and toxinotypes have been reported
- Genome plasticity of *C. difficile* is high and mobile genetic elements account for ~10% of the DNA (Sebaihia, 2006; Stabler, 2009; He, 2010)

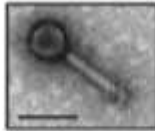
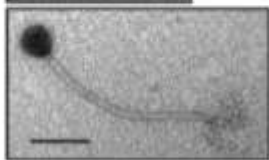
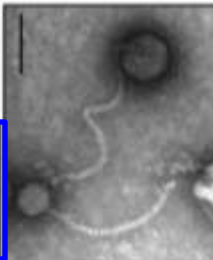


Impacts of prophages on *C. difficile*

- Prophages are intimately linked to virulence in several pathogens (Brüssow, MMBR, 2004)
- Phage ϕ CD119 was shown to decrease toxin expression in *C. difficile* (Govind, J. Virol. 2009)
- A phage island (SMPI1) was identified in the recent NAP1/027 epidemic clone R20291 (Stabler, Gen. Biol. 2009)

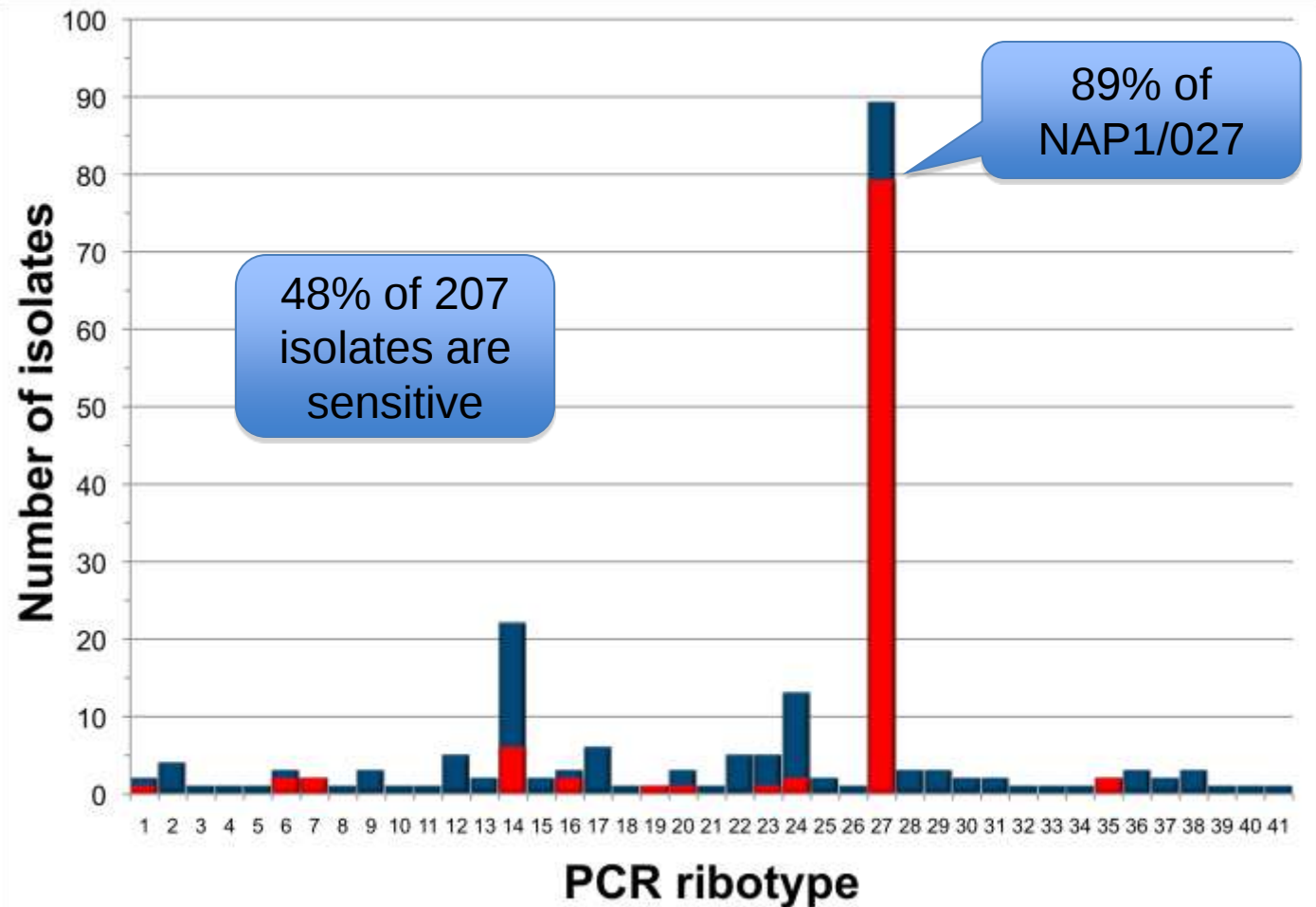


Temperate phages induced from clinical isolates

Phage or particles	Family	Capsid diam (nm) ^a	Tail length (nm) ^a	Tail diam (nm) ^a	Electron microscopy image ^b
φCD5	<i>Myoviridae</i>	66	127	18	
φCD8-1	<i>Siphoviridae</i>	90	432	12	
φCD8-2	<i>Siphoviridae</i>	46	338	11	
φCD24	<i>Siphoviridae</i>	66	302	10	
φCD38-1	<i>Siphoviridae</i>	77	402	15	 
φCD38-2	<i>Siphoviridae</i>	58	262	12	
φCD52	<i>Myoviridae</i>	64	125	19	
φCD630	<i>Myoviridae</i>	62	126	19	
PT-LPs			130	18	

(Fortier, AEM 2007)

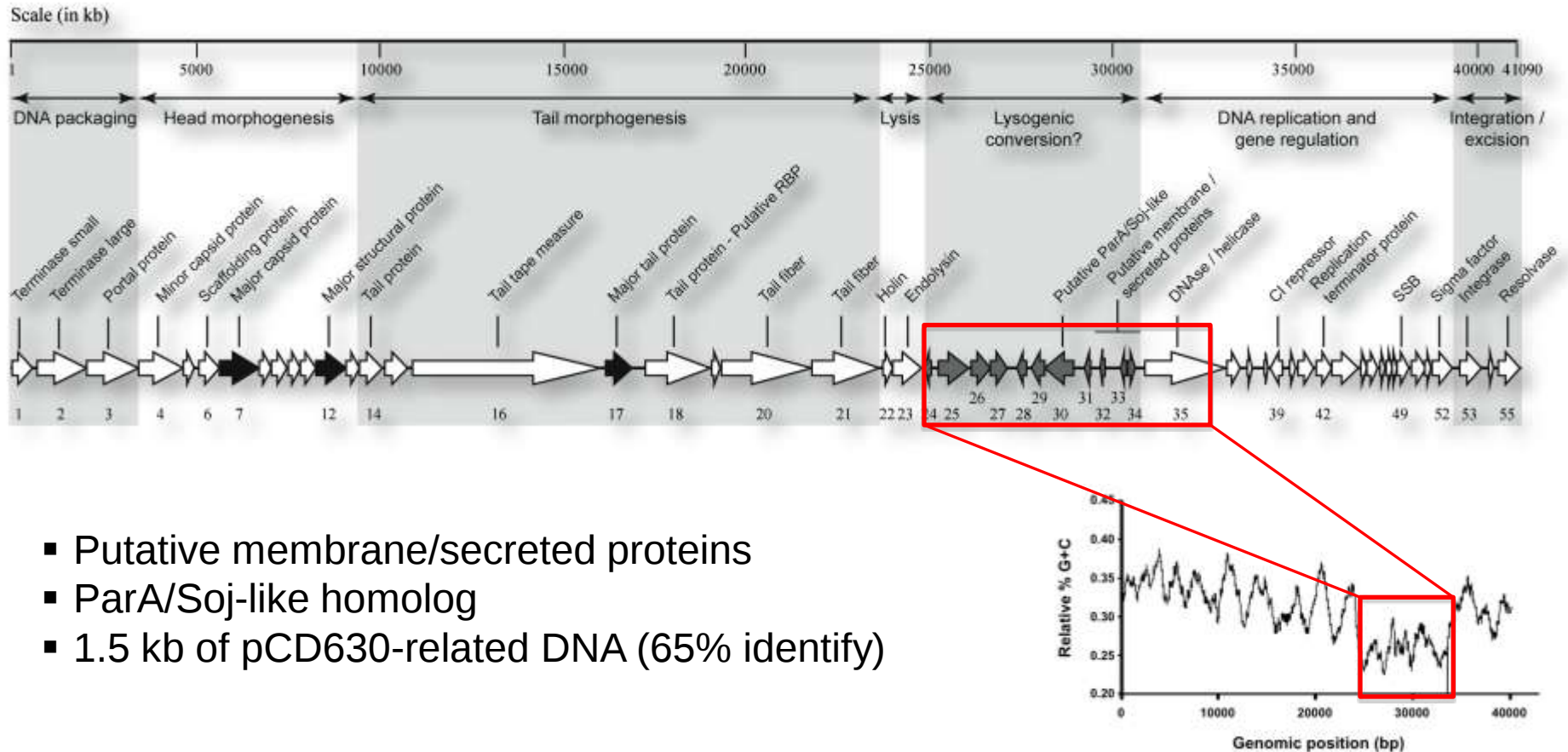
Host range of ϕ CD38-2



(Sekulovic, under revision)



Genome sequence of ϕ CD38-2



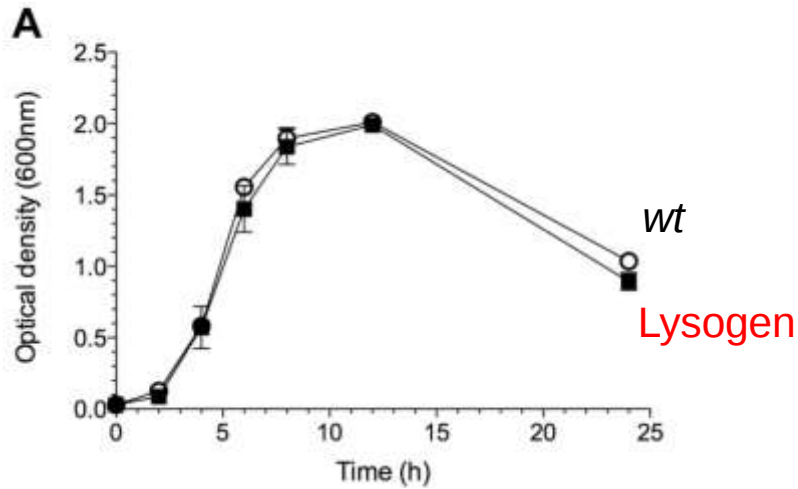
- Putative membrane/secreted proteins
- ParA/Soj-like homolog
- 1.5 kb of pCD630-related DNA (65% identify)

Recent recombination with a plasmid?

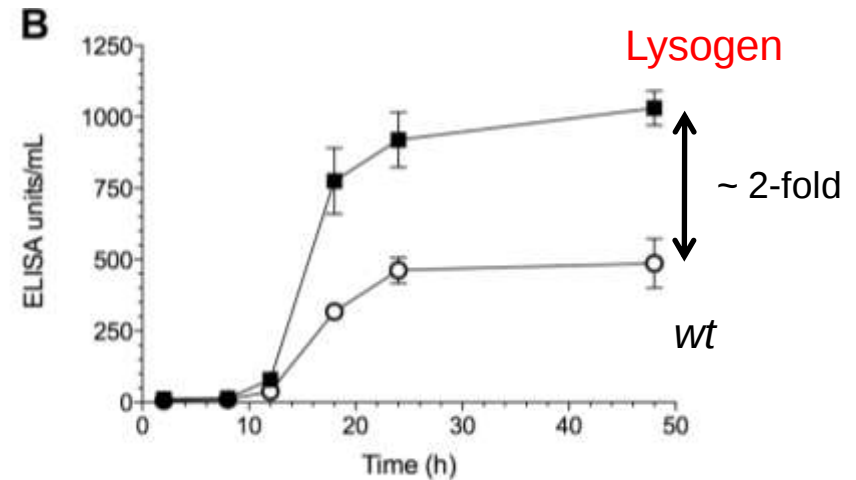
(Sekulovic, under revision)

Phenotype of NAP1/027 lysogens carrying ϕ CD38-2

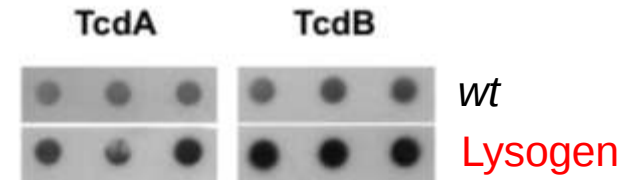
Growth



Toxins in culture supernatants
(ELISA assay)



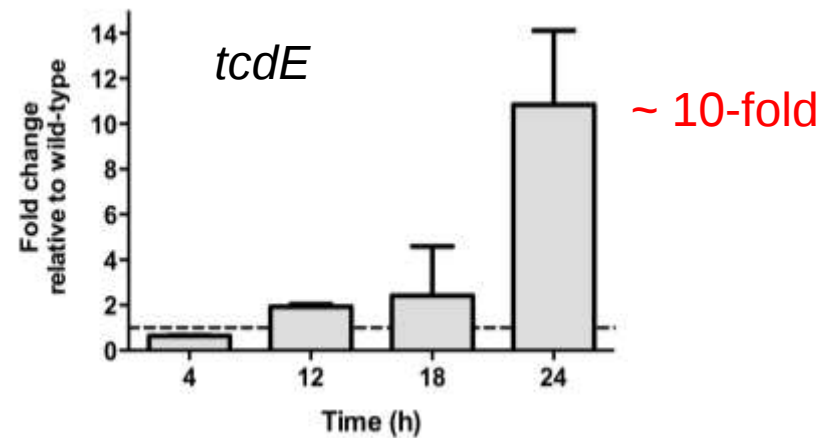
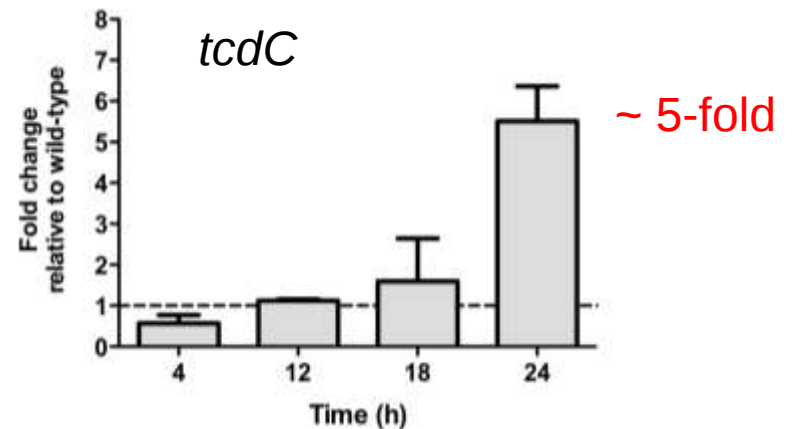
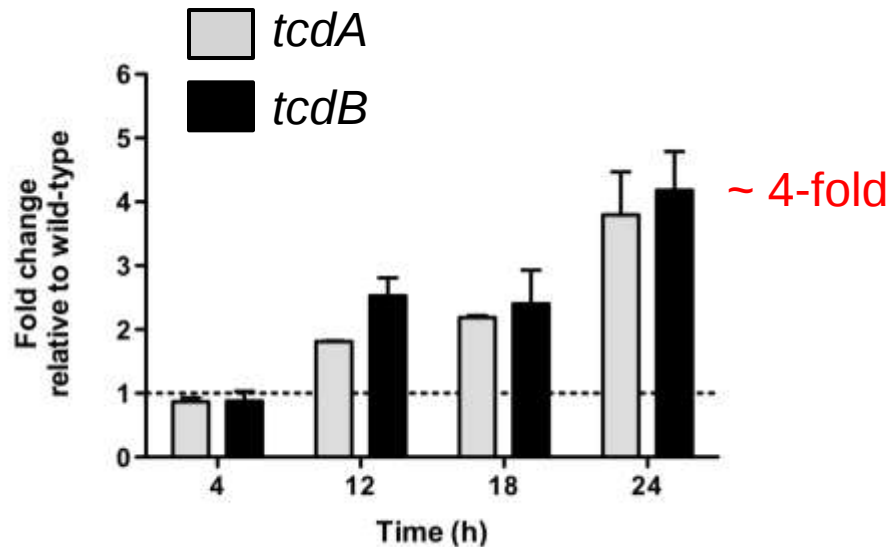
ϕ CD38-2 stimulates toxin production



(Immunodot blot at 24h)

(Sekulovic, under revision)

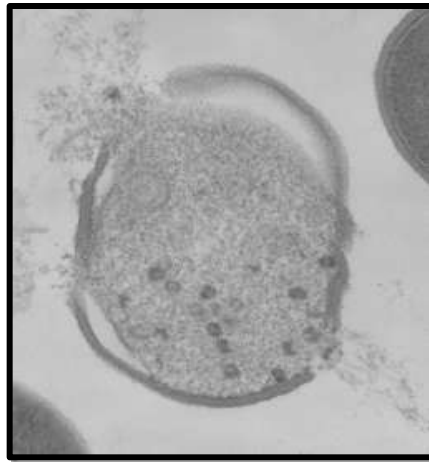
Quantification of PaLoc mRNA levels in lysogens vs wild-type



(Sekulovic, under revision)

Further evidence that prophages affect toxin expression

Phage therapy against *C. difficile*?



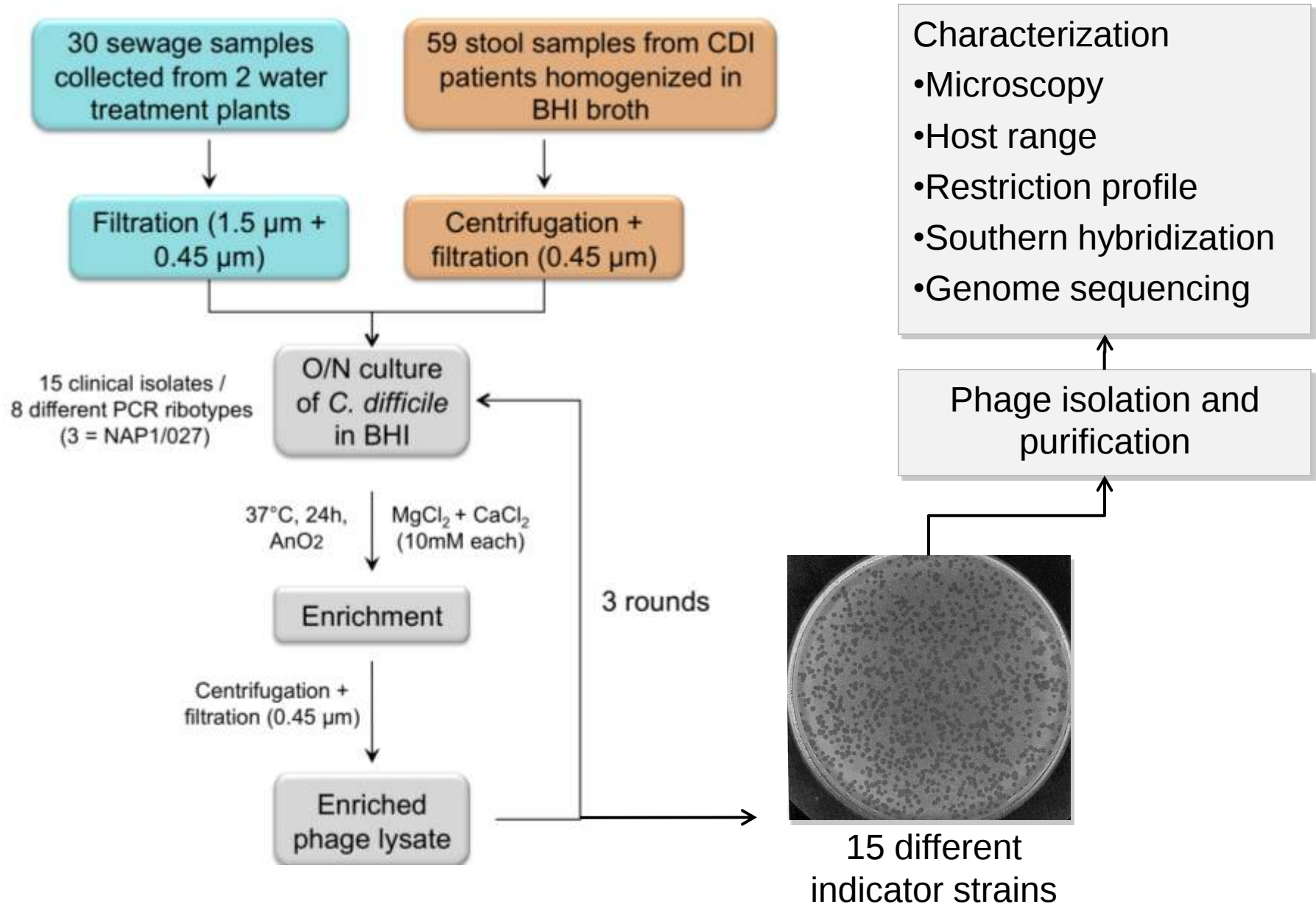
Phage therapy against *C. difficile*?

- Treatment of CDI is not always straightforward with current antibiotics
 - Frequent relapses and recurrent infections
- Need for new therapeutic solutions

Renewed interest in phage therapy
using virulent phages (i.e. strictly lytic)

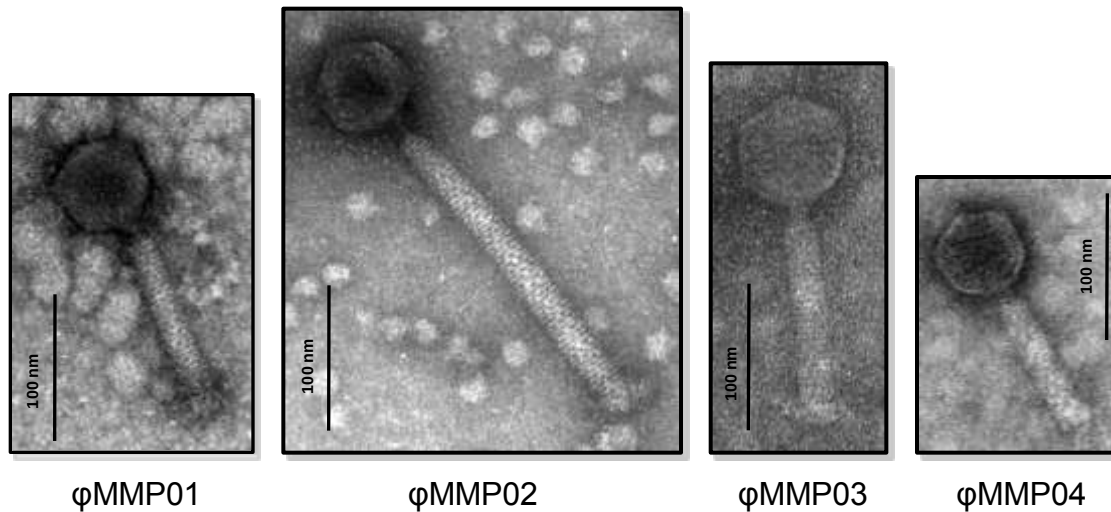


Searching for virulent phages...

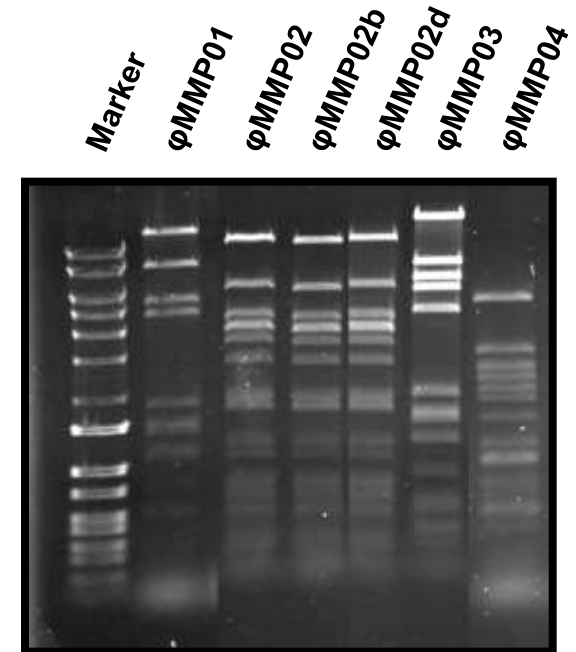


Phages were isolated from human feces

6/59 patients carried free phage particles in their feces
(0/30 positive sewage samples)

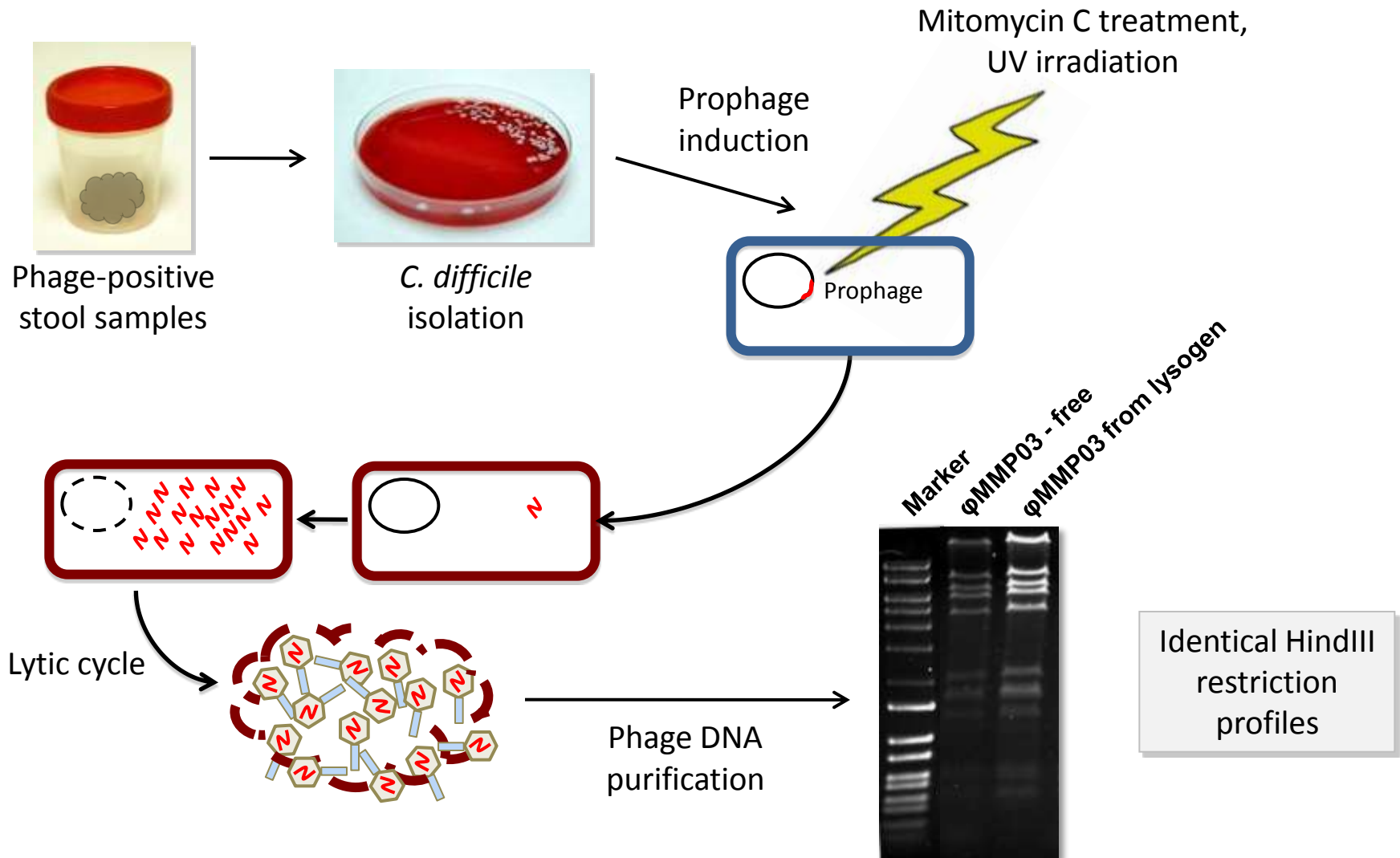


HindIII restriction profile



All phages were temperate and were able to lysogenize their host...

Phages were released from *C. difficile* in vivo



No virulent phages suitable for therapy but...

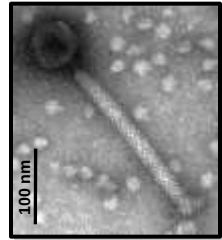
In vivo prophage induction occurred spontaneously during CDI

Spontaneous induction may have various impacts:

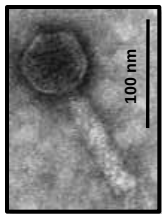
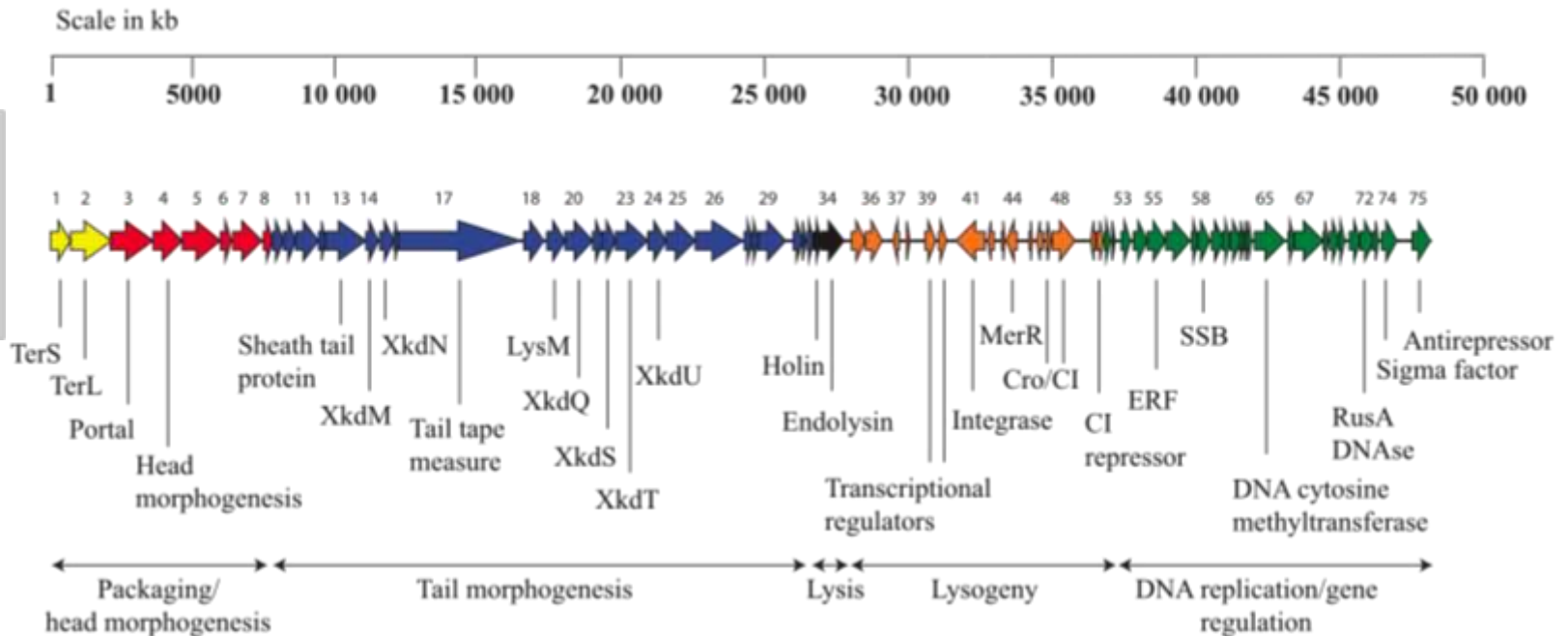
- Lysis of competing strains (during multiple infections)
- Higher toxin release upon cell lysis
- Other impacts?



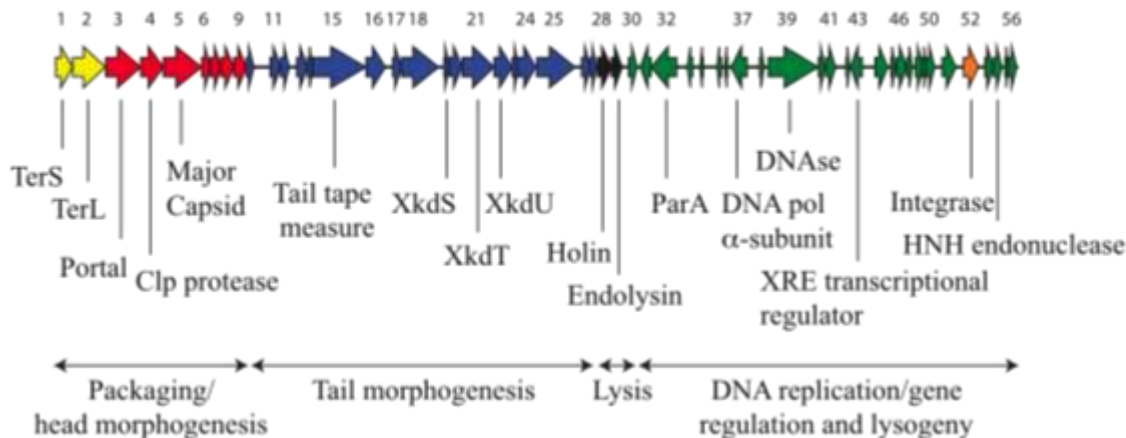
ϕ MMP02 and ϕ MMP04 genome sequence



ϕ MMP02
(48,396 bp)



ϕ MMP04
(31,662 bp)



No toxin or
virulence
factor
detected

Take home message...

- We provide further evidence that **prophages participate in the regulation of toxin expression**
 - The already “hypervirulent” NAP1/027 clone can become more virulent through the acquisition of specific prophages
 - Could other virulence characteristics be affected by prophages? (e.g. adhesion, sporulation)



Take home message...

- Prophage induction is probably frequent during CDI
 - Higher infection rates = higher phage mobility = faster bacterial evolution
 - Antibiotics could potentially stimulate prophage induction



Acknowledgements

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