

Inactivation of *stx*-phages by probiotic *E. coli* strain Nissle 1917

04.06.2019, Ghent

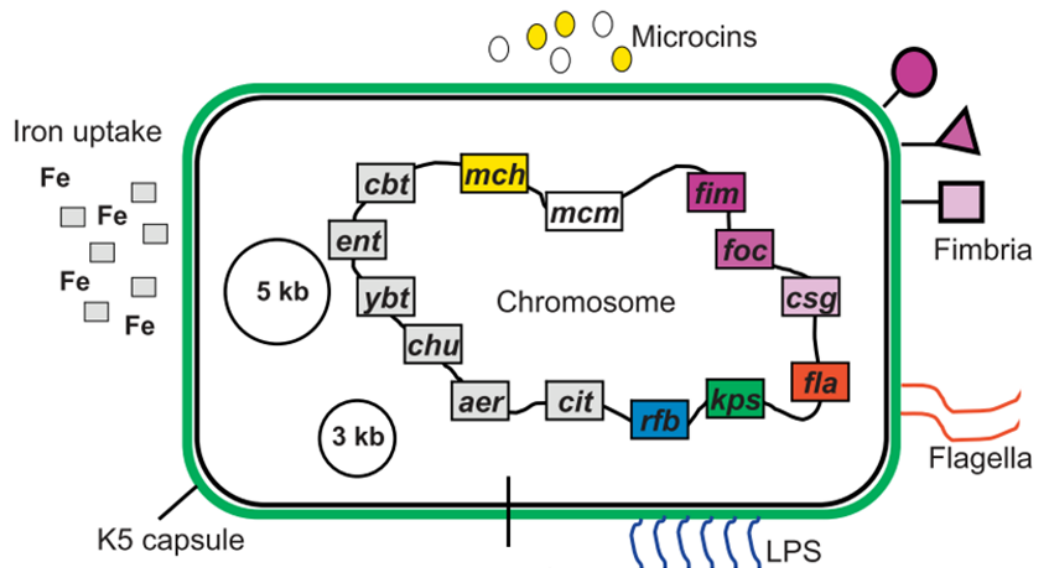
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Introduction

E. coli Nissle 1917 (EcN) - probiotic bacteria; non-pathogenic strain; O6:K5:H1

- Active component of the gastro-intestinal medication (Mutaflor®)



Adapted from Jacobi et al., Dig Dis 2011;29:600–607

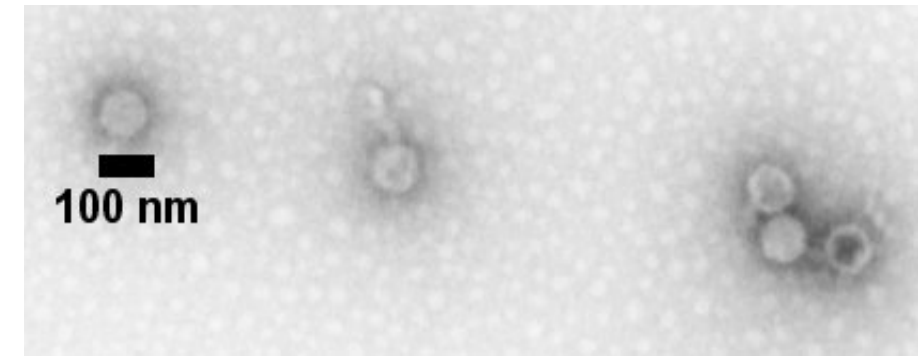
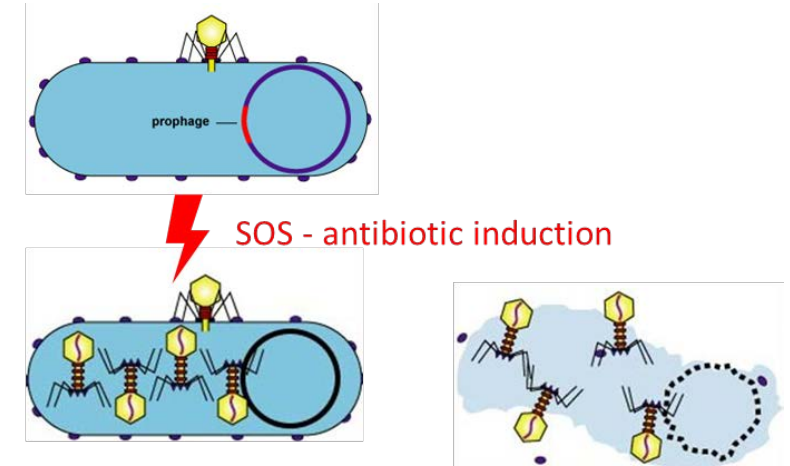
- **Treatment:** Inflammatory Bowel Disease (IBD) (Schultz et al. 2008), Diarrhoea (Henker et al. 2008)
- **Antagonistic activity:** *Salmonella*, *Shigella*, *Candida albicans*, *Vibrio cholerae* (Altenhoefer et al. 2004, Reissbrodt R. et al. 2009)
- Reduces the shigatoxin level and growth of EHEC stains up to 90 % when cocultured (Rund S. et al. 2013, Mohsin et al. 2015)

Introduction

- EHEC strains harbour *stx*-phages as prophage in their genome
- Upon induction, the phages are produced and can infect other bacteria and turn the newly formed lysogens into Stx producers

Stx-phages

- Lambdoid family of bacteriophages
- Short tailed phages (~10 nm long) with an icosahedral head (~100 nm wide) (Mondal et al. 2016)
- Harbour *stx* genes downstream of the Antiterminator *Q*
- Have been detected in cattle feces, river water and sewage



stx-phages (transmission electron microscope)
Bury S., PhD thesis

Objective-1

1. Stx-phage sensitivity of EcN

To investigate whether *stx*-phages are capable of turning the probiotic EcN into a Stx producing lysogen

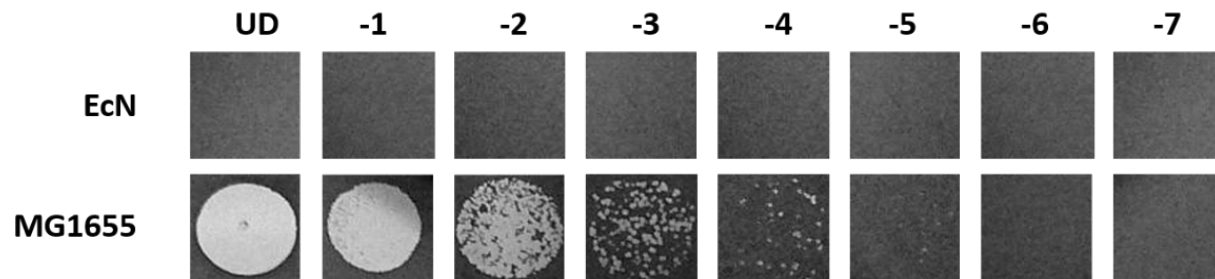
***Stx*-phage sensitivity of EcN**

1. Are *stx*-phages able to lyse or lysogenize EcN?

Stx-phage sensitivity of EcN

1. Phage-Plaque-Assay with EcN or *E. coli* K-12 MG1655 as indicator strain

- Use EcN and MG1655 as indicator strains on a Phage-Plaque-Assay (PPA)
- Drop serial dilutions of *stx*-phages on top of the plates
- Determine the lysis after an over night incubation at 37 °C



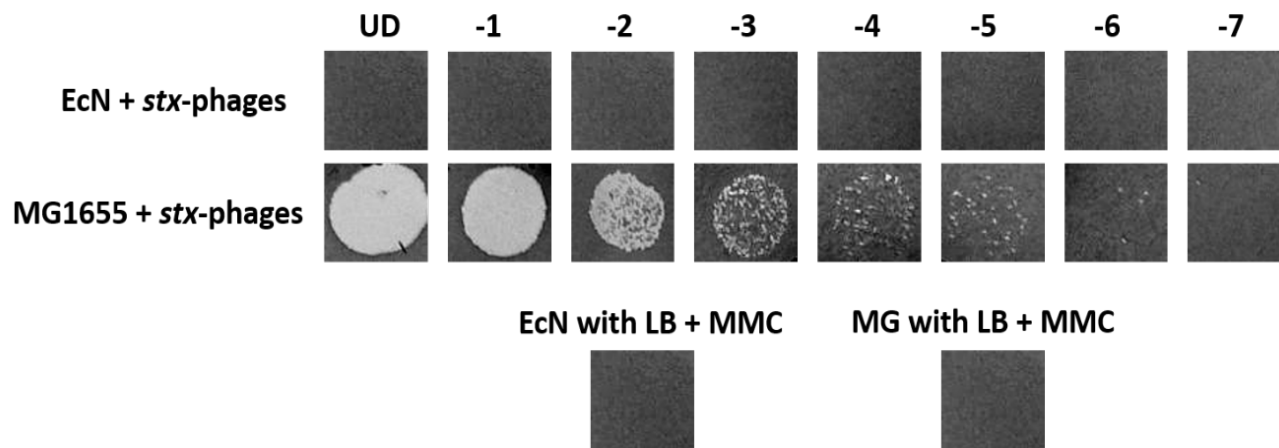
EcN was not lysed by *stx*-phages

Stx-phage sensitivity of EcN

2. Detection of lysogenic EcN

A. Phage plaque assay

1. 48 h, static incubation of *E. coli* +/- stx-phages (1:1)
2. Wash *E. coli* (2 x)
3. Over night phage induction of lysogens with 1 µg/ml Mitomycin C
4. Enhance phage signal by incubating the supernatant with MG1655
5. PPA



B. PCR

1. 24 h, static incubation of *E. coli* +/- stx-phages (100:1)
2. Plate *E. coli* on LB-Agar plates
3. Collect colonies for a stx-phage specific PCR



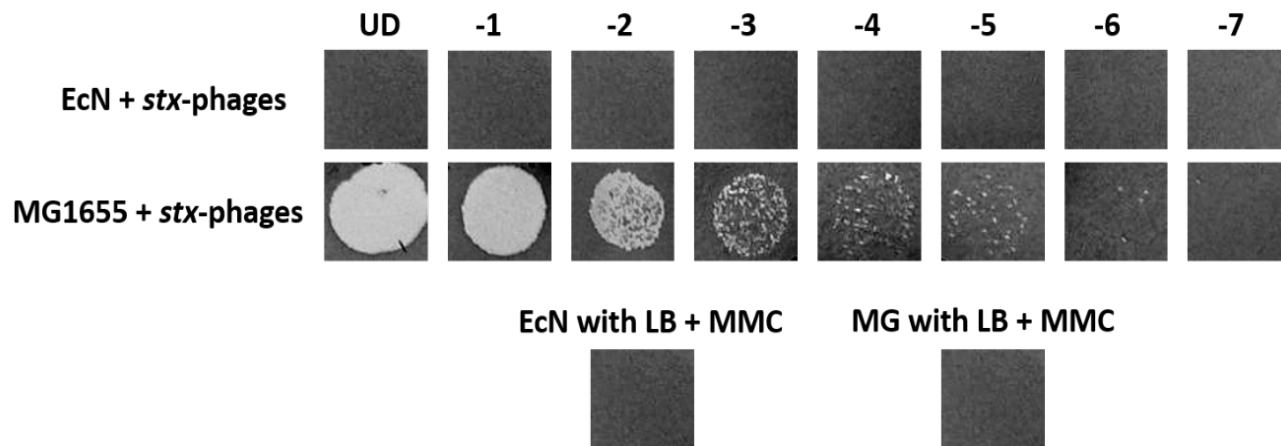
stx2 amplicon: 518 bp

Stx-phage sensitivity of EcN

2. Detection of lysogenic EcN

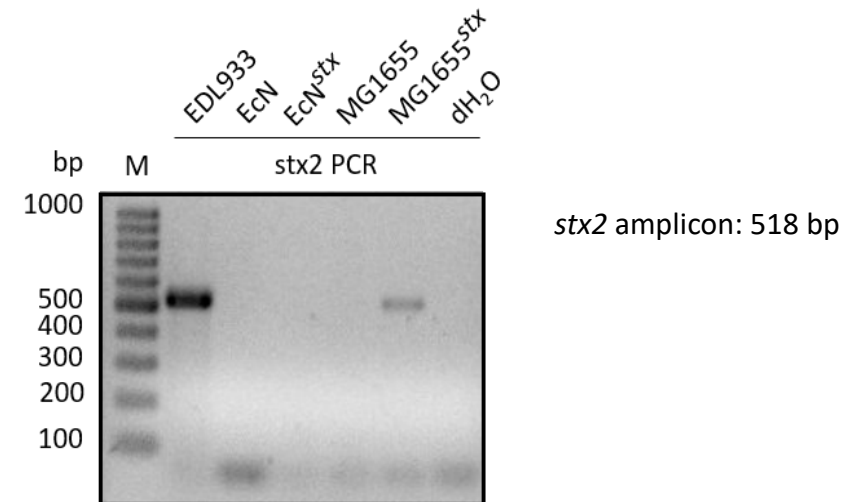
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B. PCR

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Stx-phage sensitivity of EcN

2. Detection of lysogenic EcN

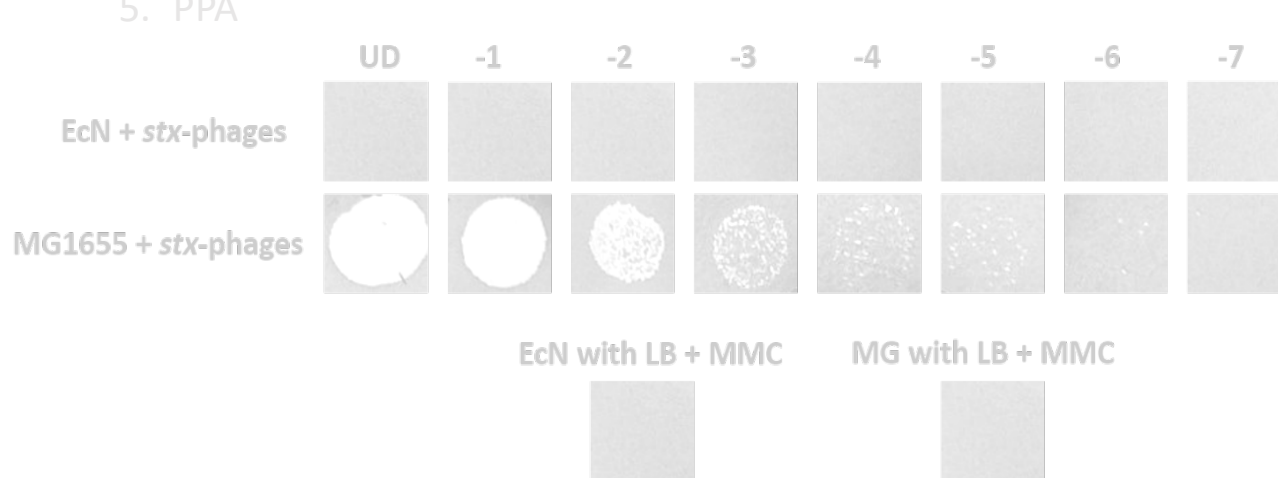
A. Phage plaque assay

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2. Wash *E. coli* (2 x)
3. Over night p
4. Enhance pha
5. PPA

B. PCR

1. 24 h, static incubation of *E. coli* +/- stx-phages (100:1)

No lysogenic EcN could be detected!



stx2 amplicon: 518 bp

***Stx*-phage sensitivity of EcN**

1. Are *stx*-phages able to lyse or lysogenize EcN?

- Neither were the phages able to lyse nor to lysogenize EcN

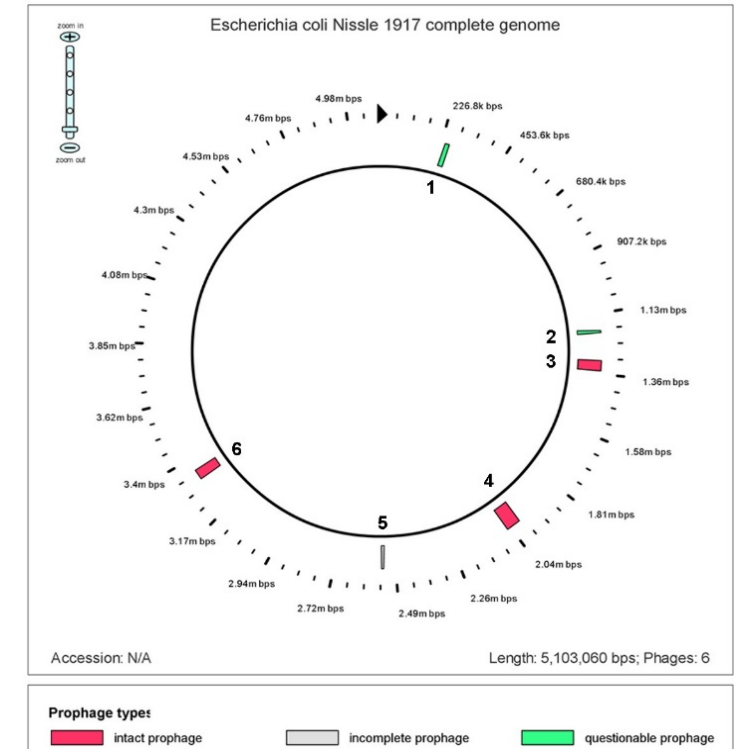
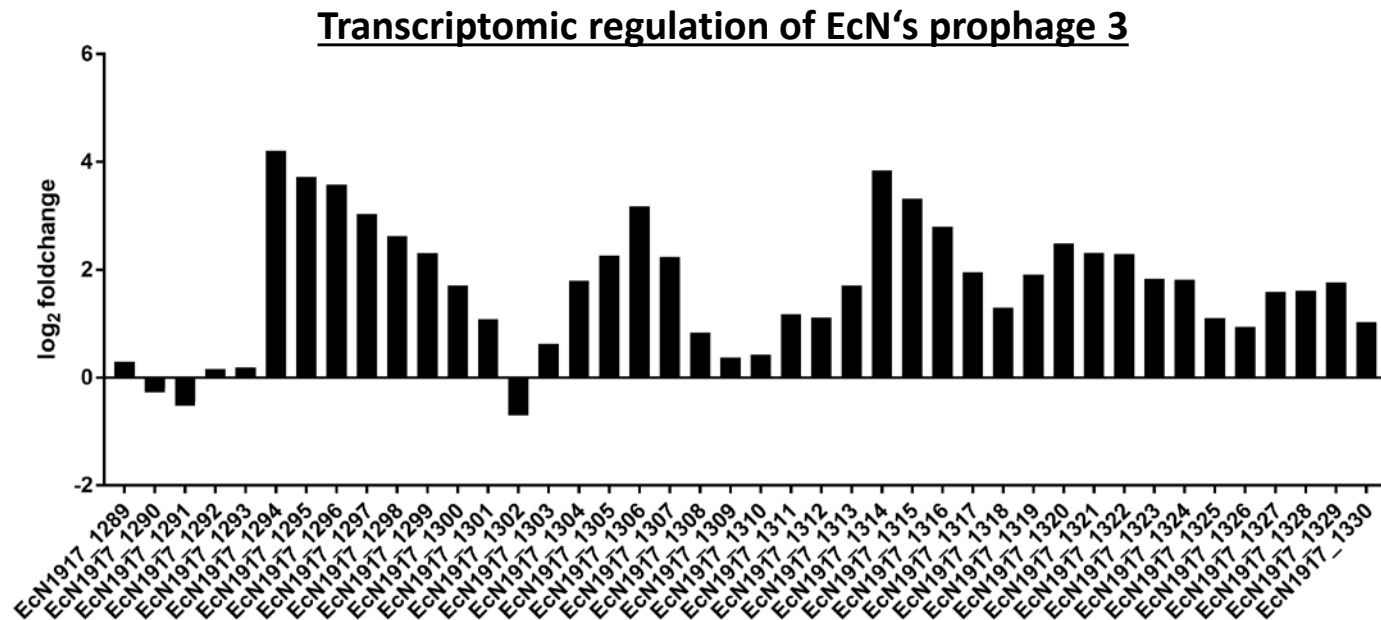
Stx-phage sensitivity of EcN

1. Are *stx*-phages able to lyse or lysogenize EcN?
 - Neither were the phages able to lyse nor to lysogenize EcN
2. How does EcN protect itself from *stx*-phage attacks?

Stx-phage sensitivity of EcN

Transcriptome analysis of EcN +/- stx-phages

- Isolate the RNA from EcN +/- stx-phages (100:1) after 16 h of incubation
- Strong upregulation of a 39.8 kbp long lambdoid prophage of EcN in the presence of stx-phages



Prophages predicted in the genome of EcN by PHAST

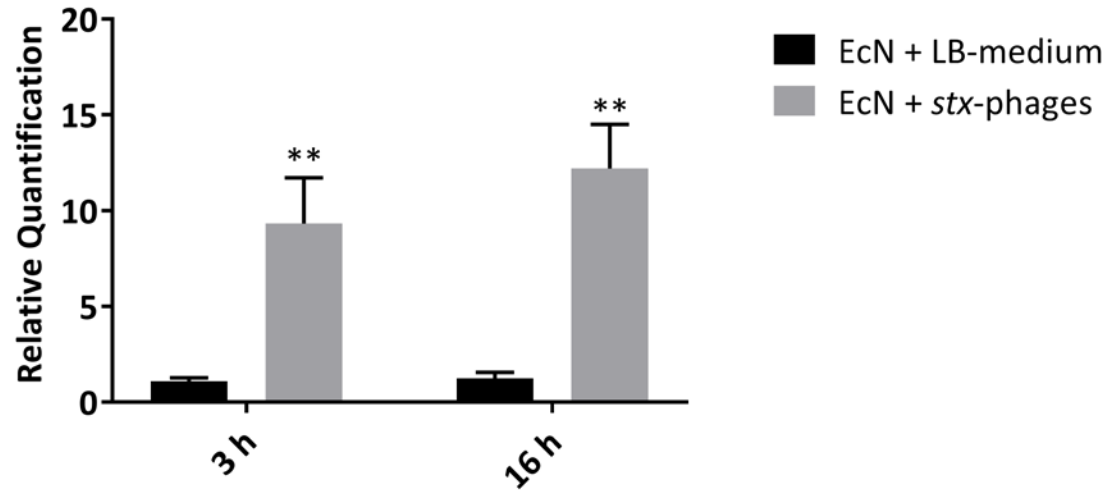
Bury, Soundararajan et al. (2018). [Front Microbiol](#)

Candidate gene identification

➤ EcN_1294 – phage repressor gene (*pr*)

Alvarez et al., 1999: Stable expression of the *Lactobacillus casei* bacteriophage A repressor blocks phage propagation during milk fermentation

➤ qRT-PCR confirmation of the *pr* upregulation in EcN +/- *stx*-phages

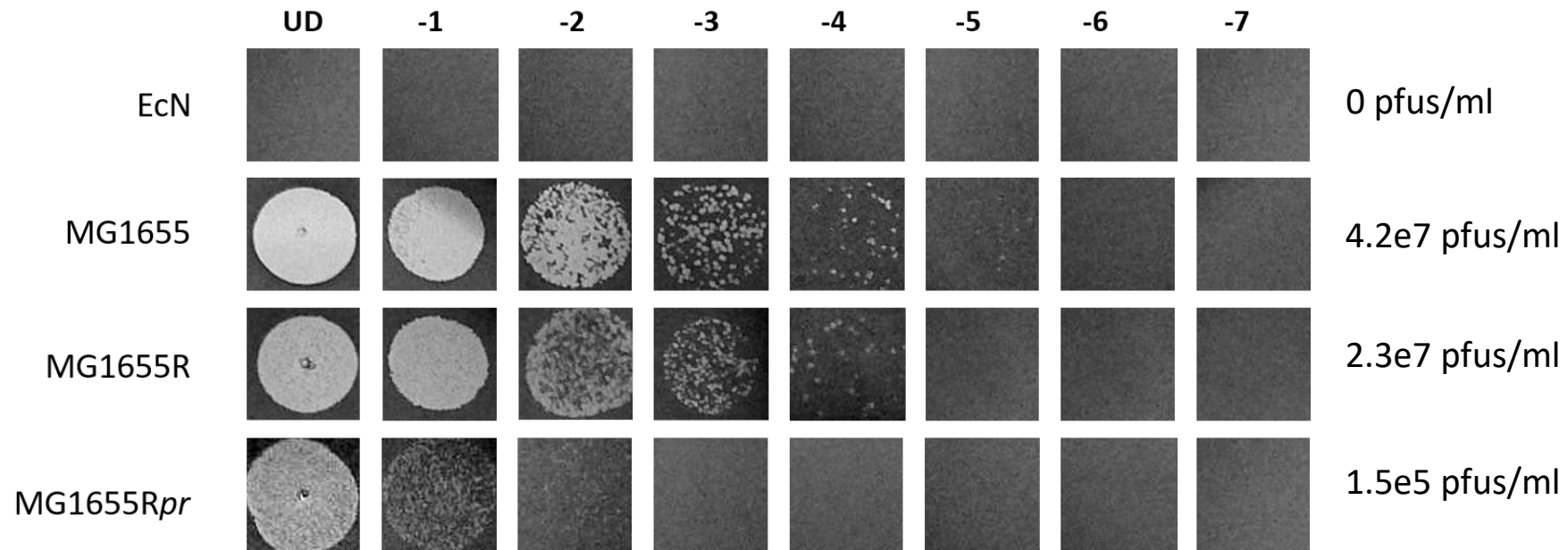


Bury, Soundararajan et al. (2018). [Front Microbiol](#)

Candidate gene identification

Can prophage genes of EcN protect MG1655?

- Serial dilutions of *stx*-phages on bacterial lawns of EcN, MG1655 or the recombinant MG1655 strains that contains pUC19 (MG1655R), pUC19_pr (MG1655pr)



Bury, Soundararajan et al. (2018). [Front Microbiol](#)

Stx-phage sensitivity of EcN

1. Are *stx*-phages able to lyse or lysogenize EcN?

- Neither were the phages able to lyse nor to lysogenize EcN

2. How does EcN protect itself from *stx*-phage attacks?

- The **phage repressor gene** in the lambdoid prophage of EcN is involved in the protection of EcN towards a *stx*-phage infection

Objective-2

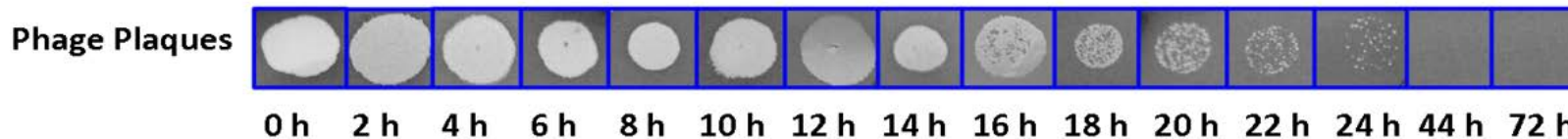
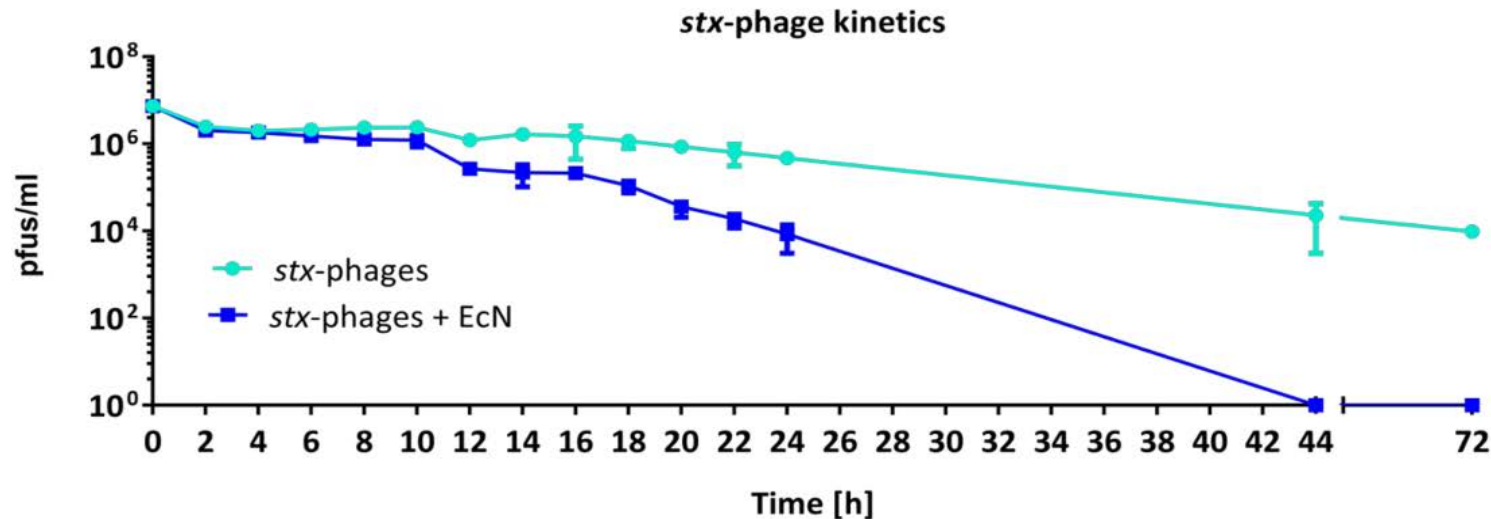
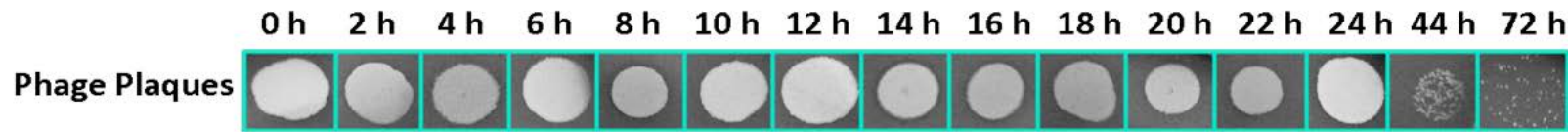
2. Inactivation of *stx*-phages by EcN

To investigate the influence of EcN towards isolated *stx*-phages during coincubation

Inactivation of *stx*-phages by EcN

1. Does EcN influence the infectivity of isolated *stx*-phages?

➤ Incubate *stx*-phages +/- EcN (1:10) static at 37 °C



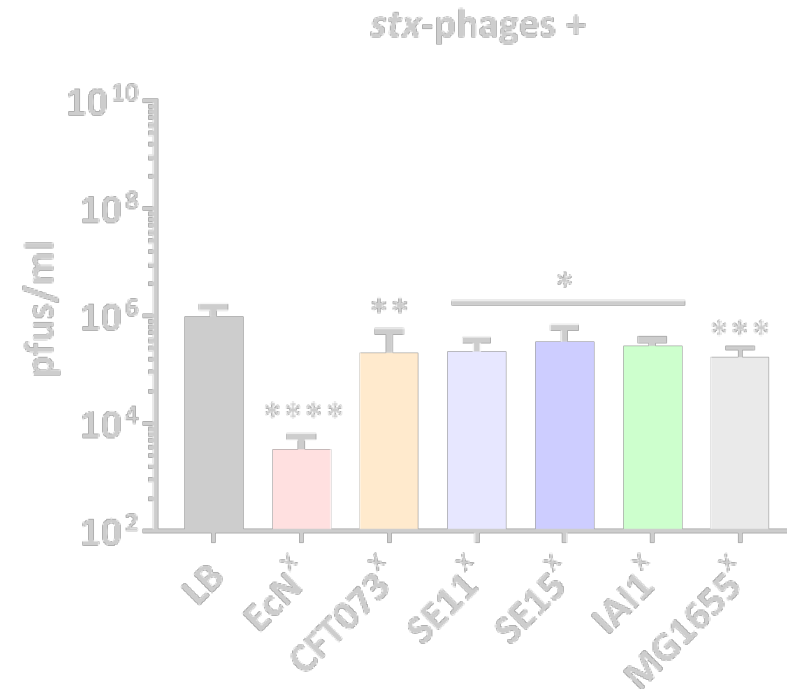
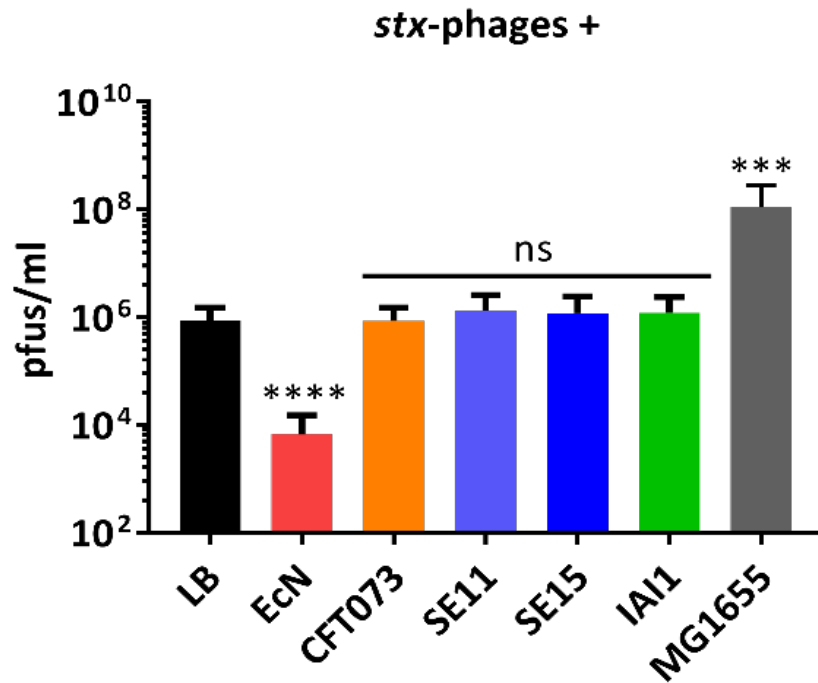
➤ EcN gradually inactivated the *stx*-phages starting in EcN's stationary growth phase and no infective phages could be detected after 44 h of incubation with EcN

Inactivation of *stx*-phages by EcN

1. Does EcN influence the infectivity of isolated *stx*-phages?
 - EcN gradually inactivated the *stx*-phages starting in EcN's stationary growth phase and no infective phages could be detected after 44 h of incubation with EcN
2. Is this phage inactivation an EcN unique attribute?

Commensal *E. coli* + *stx* -phages

Incubate *stx*-phages +/- *E. coli* (1:10) static at 37 °C for 24 h

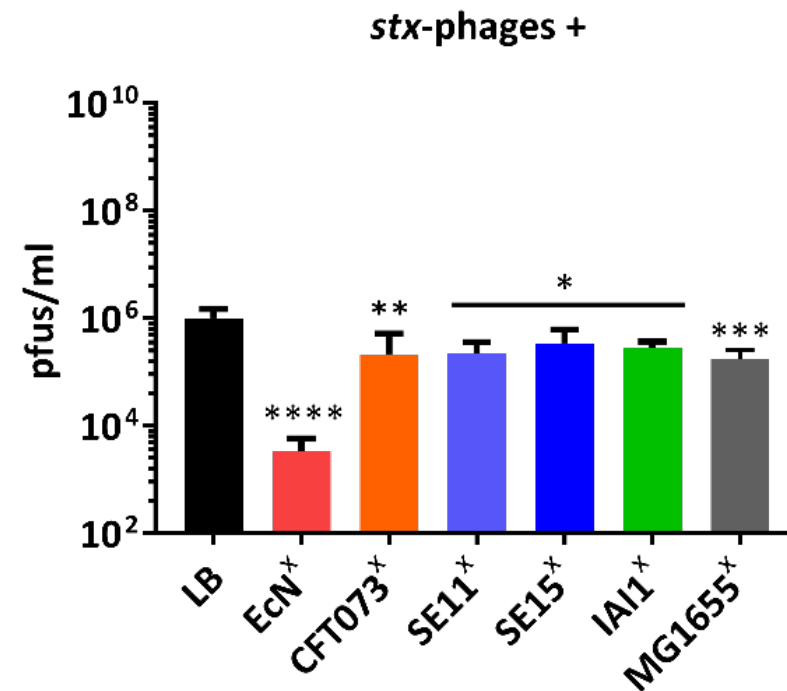
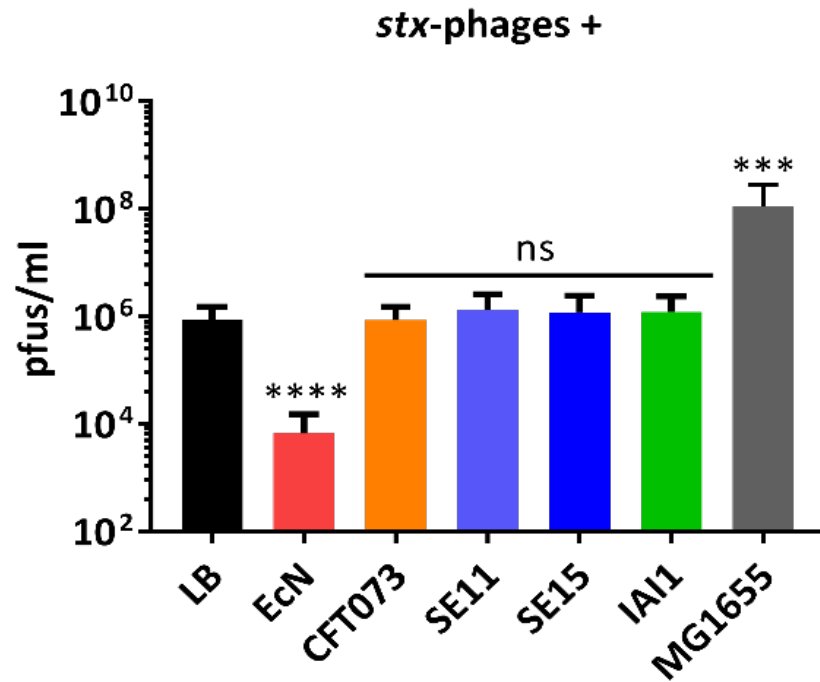


CFT073 – Uropathogenic *E. coli*;
 SE11, SE15, IAI1 – Commensal *E. coli* isolated from healthy human

X: 1 h, 100 °C heat killed *E. coli*, CFUs: 1.6e9

Commensal *E. coli* + stx -phages

Incubate stx-phages +/- *E. coli* (1:10) static at 37 °C for 24 h



X: 1 h, 100 °C heat killed *E. coli*, CFUs: 1.6e9

Inactivation of stx-phages by EcN

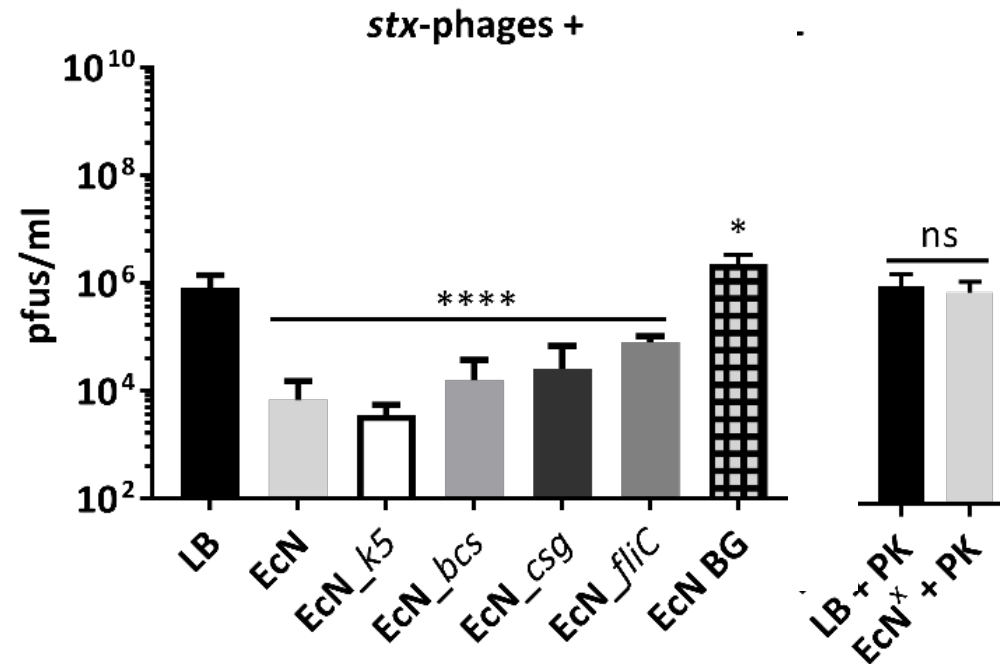
1. Does EcN influence the infectivity of isolated *stx*-phages?
 - EcN gradually reduced the infectivity of *stx*-phages towards MG1655 starting in EcN's stationary growth phase
2. Is this phage inactivation an EcN unique attribute?
 - In contrast to EcN, other commensal *E. coli* tested were not able to inactivate the *stx*-phages
 - Factor in EcN that is responsible for *stx*-phage inactivation is **thermostable**

Inactivation of stx-phages by EcN

1. Does EcN influence the infectivity of isolated *stx*-phages?
 - EcN gradually reduced the infectivity of *stx*-phages towards MG1655 starting in EcN's stationary growth phase
2. Is this phage inactivation an EcN unique attribute?
 - In contrast to EcN, other commensal *E. coli* tested were not able to inactivate the *stx*-phages
 - Even the heat killed EcN inactivated the *stx*-phages like the living control
3. How does EcN inactivate the *stx*-phages?

Screening EcN surface mutants

Incubate *stx*-phages +/- *E. coli* (1:10) static at 37 °C for 24 h



EcN_k5: capsule mutant; EcN_bcs: cellulose mutant,
EcN_csg: curli mutant; EcN_fliC: flagellin mutant,
BG: Bacterial ghosts of EcN

Source: Prof.Dr. Werner Lubitz, Vienna

X: 1 h, 100 °C heat killed *E. coli*, CFUs: 1.6e9,
PK: Proteinase K (1 mg/ml)

Inactivation of stx-phages by EcN

1. Does EcN influence the infectivity of isolated *stx*-phages?
 - EcN gradually reduced the infectivity of *stx*-phages towards MG1655 starting in EcN's stationary growth phase
2. Is this phage inactivation an EcN unique attribute?
 - Other commensal *E. coli* tested were not able to reduce the phage infectivity as EcN
 - Heat killed EcN were still able to inactivate the *stx*-phages like the living control
3. How does EcN inactivate the *stx*-phages?
 - All tested surface mutants were still able to inactivate the *stx*-phages
 - The bacterial ghosts of EcN could not inactivate the phages
 - The *stx*-phages seem to be inactivated by a **thermostable protein of EcN** which is bound to the surface of EcN and produced in a **later growth phase**

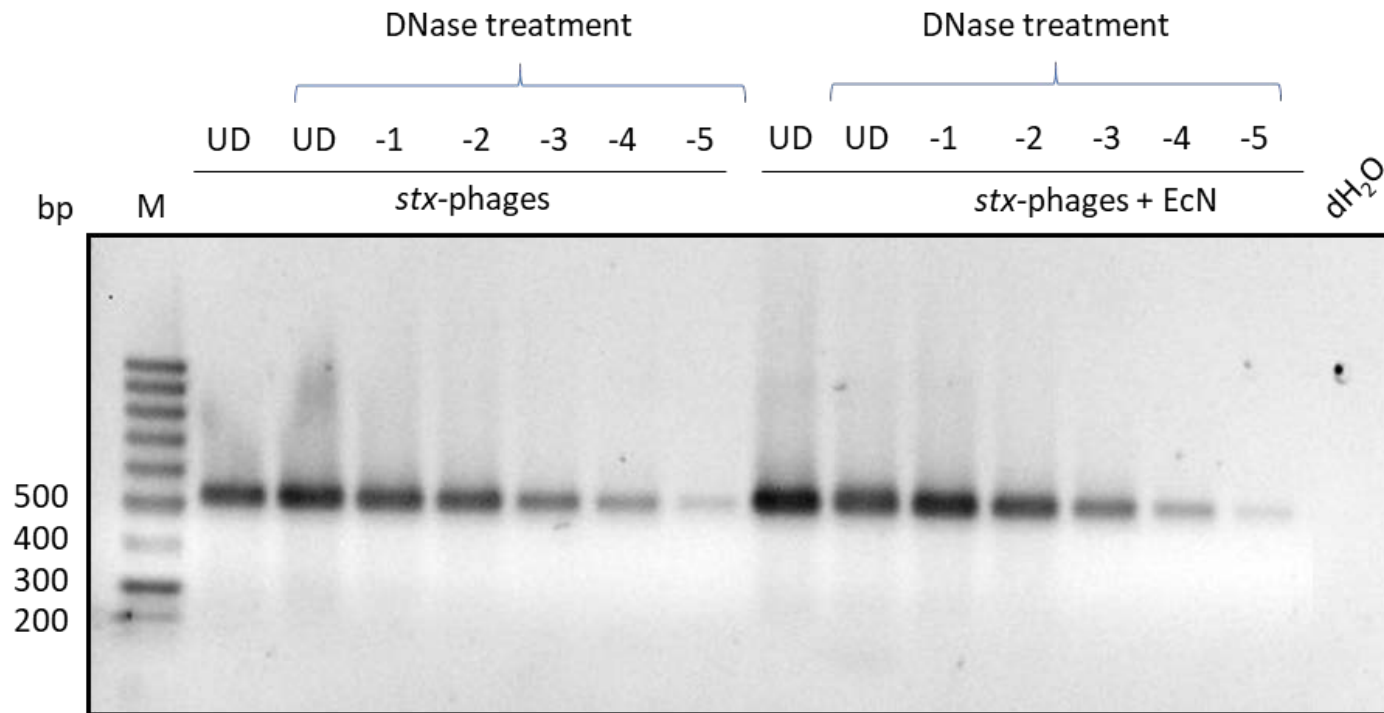
Inactivation of stx-phages by EcN

4. Does EcN destroy the phages in the coculture studies?

Influence of EcN on isolated *stx*-phages and Stx

1. 24 h incubation of isolated *stx*-phages with or without EcN

➤ *stx2* specific PCR with the sterile filtered, DNase digested supernatant



UD: undiluted, *stx2* amplicon: 518 bp

Inactivation of stx-phages by EcN

4. Does EcN destroy the phages in the coculture studies?

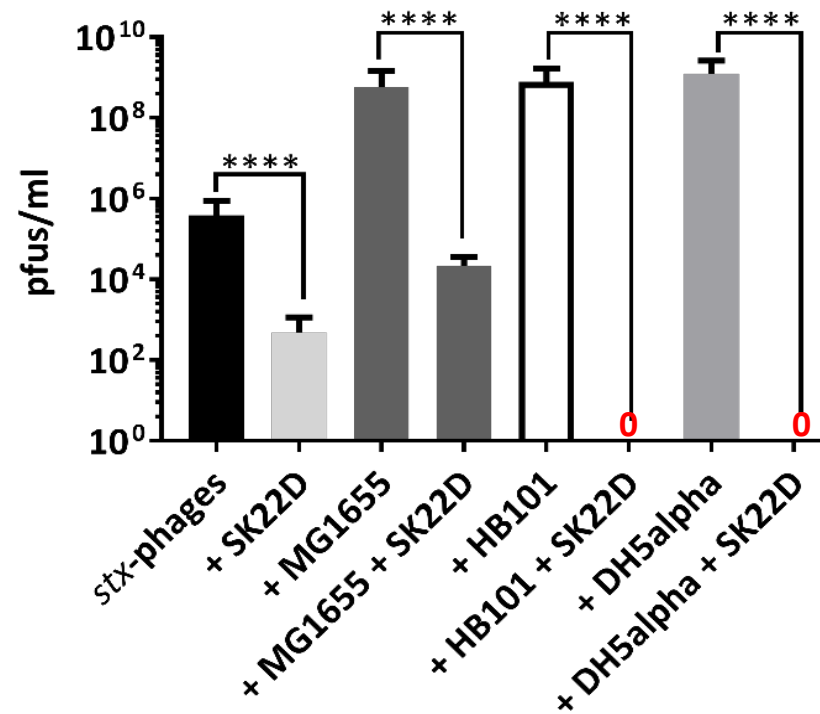
- The DNA of the *stx2*-phages was still detectable after coincubation with EcN
- EcN does not destroy the *stx*-phage DNA rather inactivates it

Objective-3

3. To examine the protection of K-12 by EcN towards *stx*-phage infection

K-12 protection

- Co-/triculture studies of isolated *stx*-phages with K-12 strains and SK22D (1:10:10), 24 h static incubation



SK22D: microcin negative mutant of EcN

Bury, Soundararajan et al. (2018). [Front Microbiol](#)

Objective-3

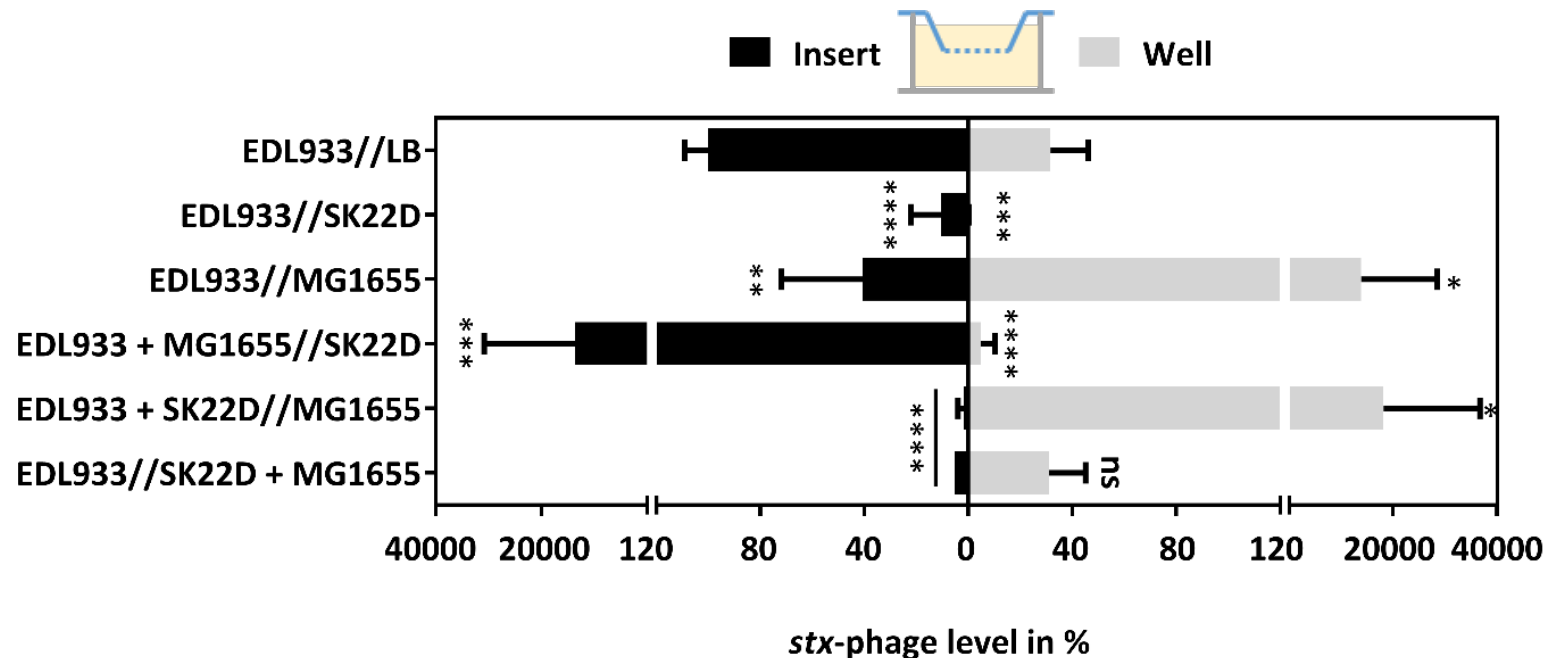
1. Can EcN influence the *stx*-phage infection of K-12 strains?
 - *stx*-phages turned all tested K-12 strains into strong *stx*-phage producers
 - EcN could interfere with this infection

EcN's influence on *stx*-phage infection of K-12 strains

1. Can EcN influence the *stx*-phage infection of K-12 strains?
 - *stx*-phages turned all tested K-12 strains into strong Stx and *stx*-phage producers
 - EcN could interfere with this infection
2. Is cell to cell contact necessary for MG1655 protection?

EcN's influence on *stx*-phage infection of K-12 strains

- The STEC strain EDL933 (insert) and SK22D, MG1655 (1:10:10) were separated in different set ups by a 0.4 µm PET Transwell membrane.



EcN's influence on *stx*-phage infection of K-12 strains

1. Can EcN influence the *stx*-phage infection of K-12 strains?
 - *stx*-phages turned all tested K-12 strains into strong Stx and *stx*-phage producers
 - EcN could interfere with this infection
2. Is cell to cell contact necessary for MG1655 protection?
 - EcN can protect MG1655 only when being cultured in the same transwell compartment

Conclusions

1. EcN cannot be infected by *stx*-phages because of its lambdoid prophage
2. A thermostable protein on the surface of EcN, synthesized in the stationary growth phase, is responsible for the *stx*-phage inactivation
3. The protection of K-12 strains by EcN is contact dependent



Thank you for the attention!

Work group

Dr. Tobias Oelschlaeger

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Members of Core Unit , Wuerzburg

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