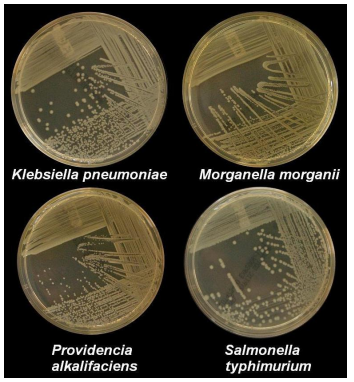
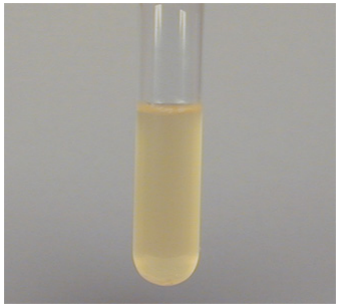
UPPSALA UNIVERSITET

Contact-Dependent Growth Inhibition (CDI) in bacteria

Sanna Koskiniemi

Bacteria are typically regarded as isolated unicellular entities

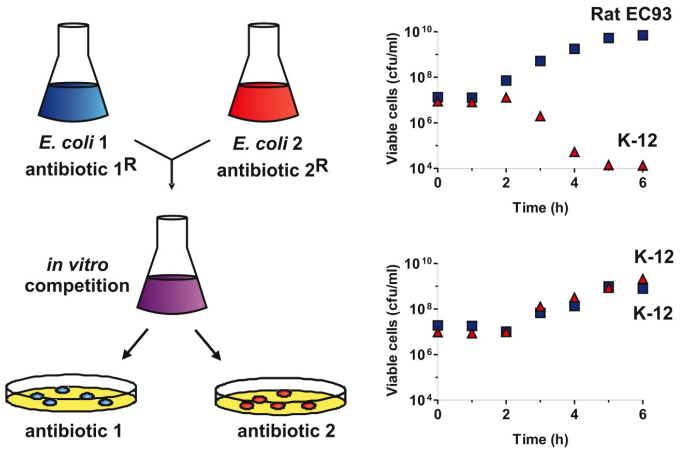




But wild bacteria often live in communities

E. coli isolate EC93 and discovery of contact-dependent growth inhibition (CDI)

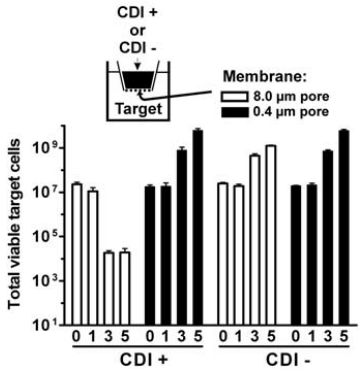
Isolate EC93 inhibits the growth of *E. coli* K-12 strains



Steph Aoki, Science 2005

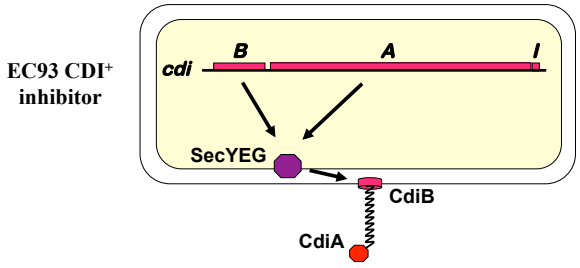
Contact-dependent Growth Inhibition

- The observed growth inhibition of lab strains of *E.coli* required cell-to-cell contact



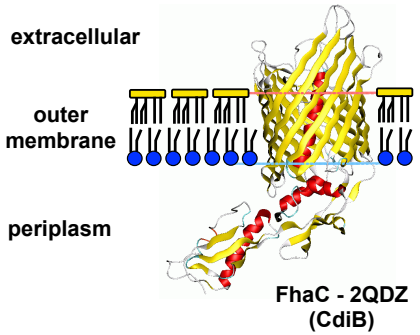
Aoki et al. 2005, Science

Contact-dependent growth inhibition (CDI)



Aoki *et al.* (2005) *Science* 309:1245-8

CdiB and CdiA are members of the two-partner secretion protein family



Clantin *et al.* (2007) *Science* 317:957-61

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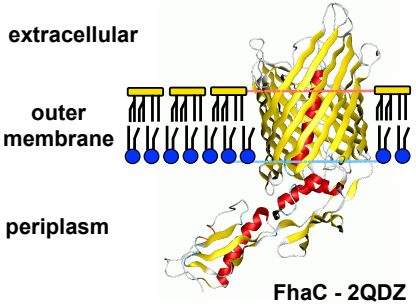
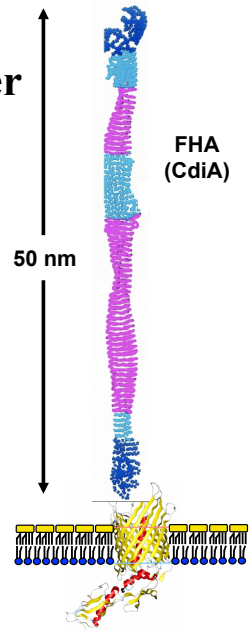
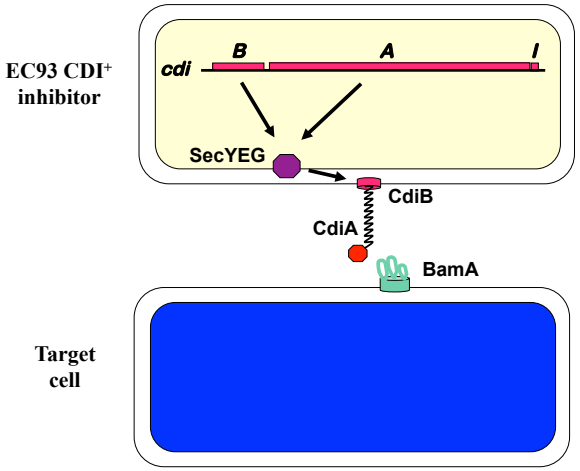


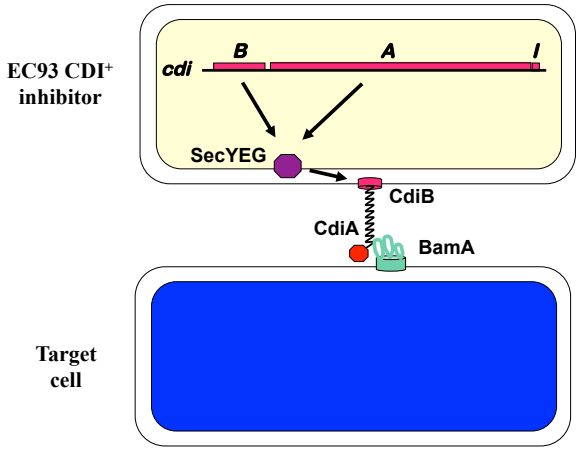


Contact-dependent growth inhibition (CDI)



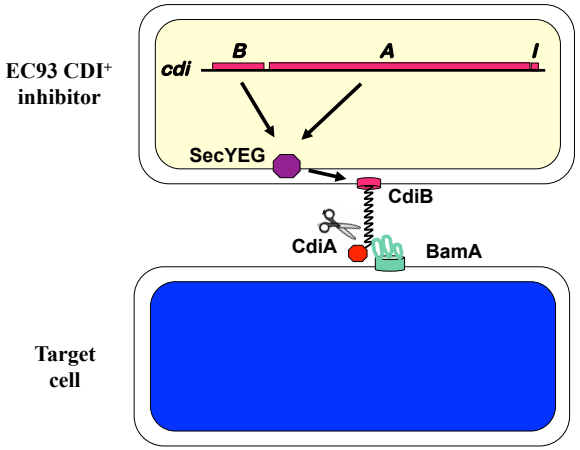
Aoki *et al.* (2008) *Mol. Micro.* 70:323-40

Contact-dependent growth inhibition (CDI)



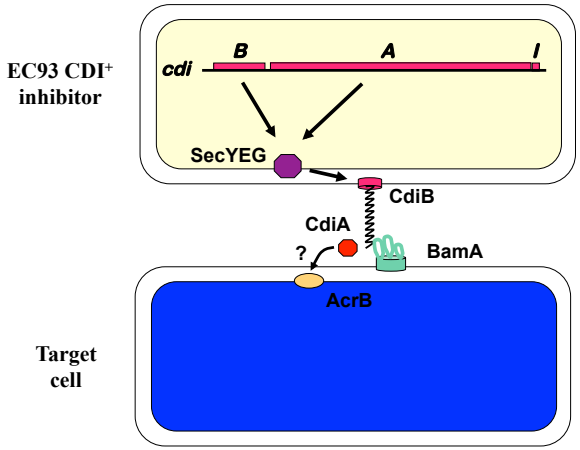
Aoki *et al.* (2008) *Mol. Microbiol.* 70:323-40

Contact-dependent growth inhibition (CDI)

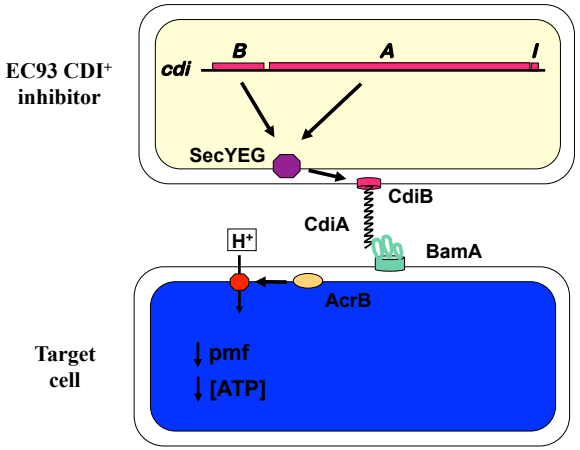


Aoki *et al.* (2008) *Mol. Microbiol.* 70:323-40

Contact-dependent growth inhibition (CDI)

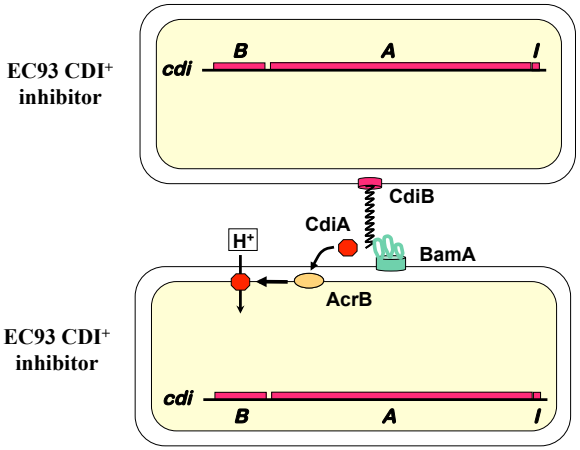


Aoki *et al.* (2008) *Mol. Microbiol.* 70:323-40



Aoki *et al.* (2009) *J. Bacteriol.* 191:1777-86

The CdiI immunity protein prevents autoinhibition



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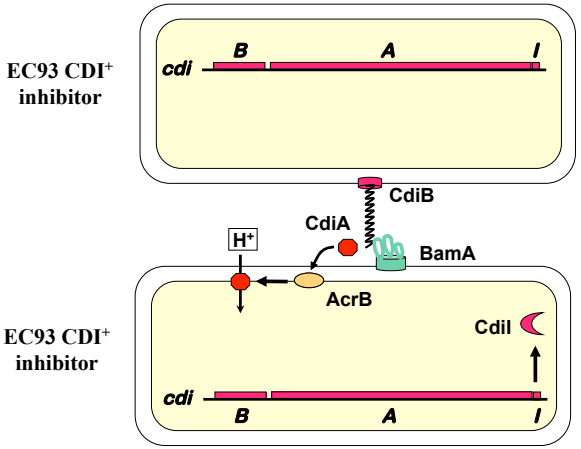

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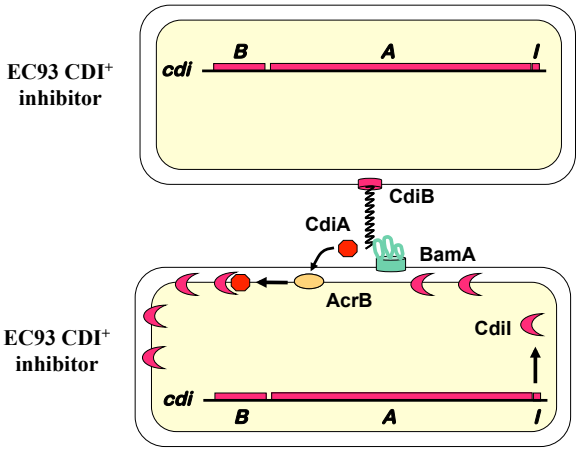
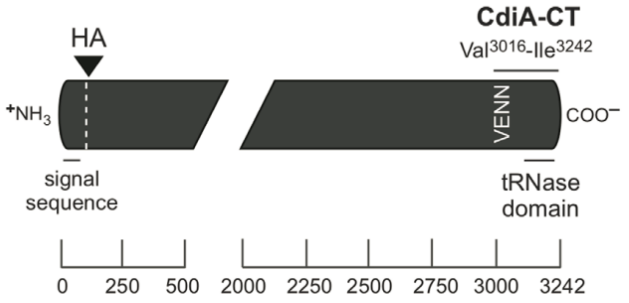
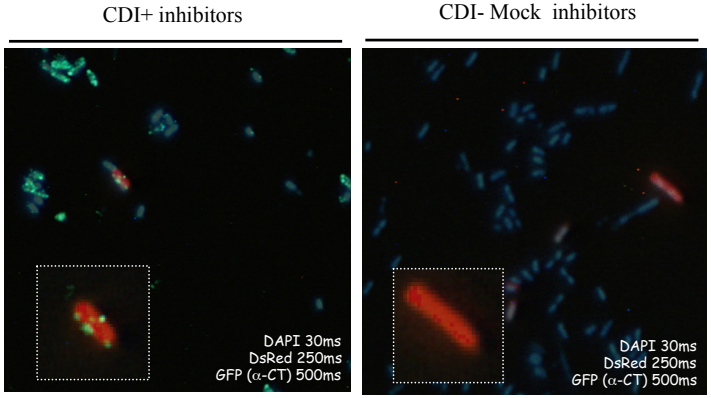


Visualization of CdiA toxin delivery into target cells - epitope tagging of CdiA^{UPEC536}



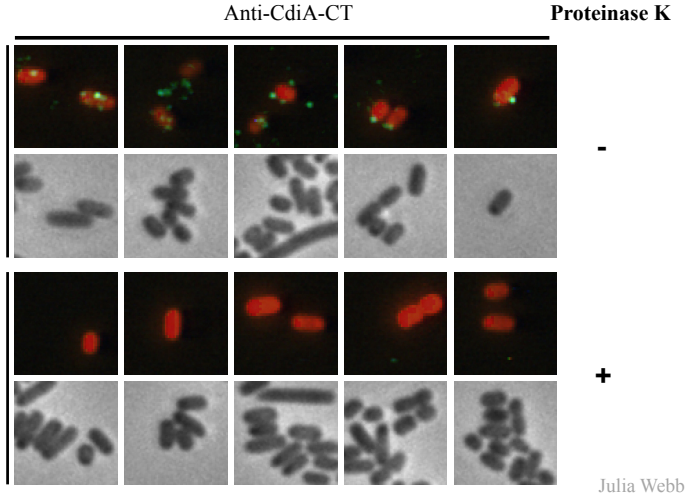
Julia Webb

CdiA-CT delivery to target cells



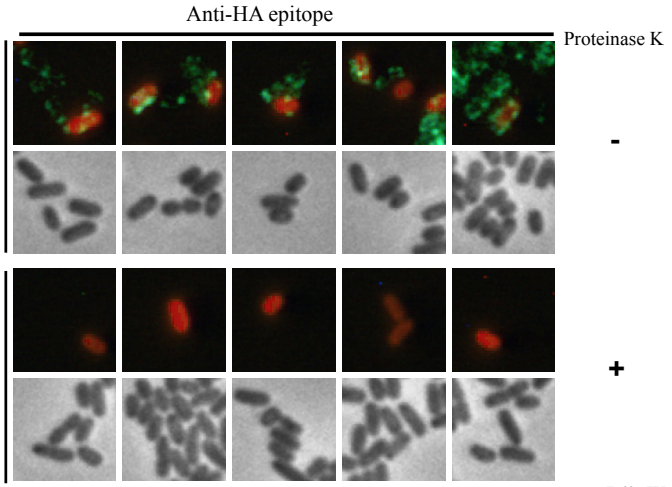
Julia Webb

CdiA-CT is delivered to the surface is proteinase K sensitive



Julia Webb

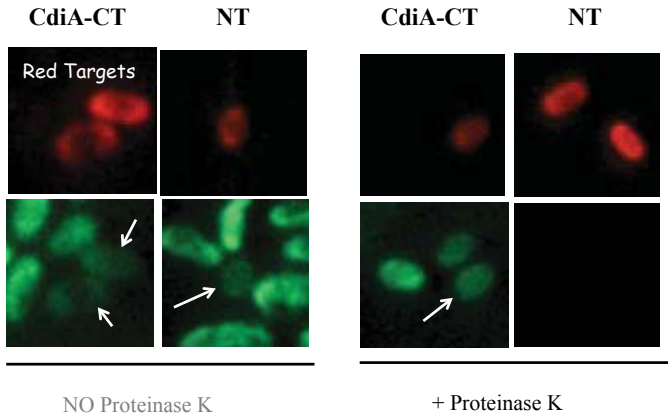
CdiA-NT is also delivered to the surface of CDI$^-$ target cells



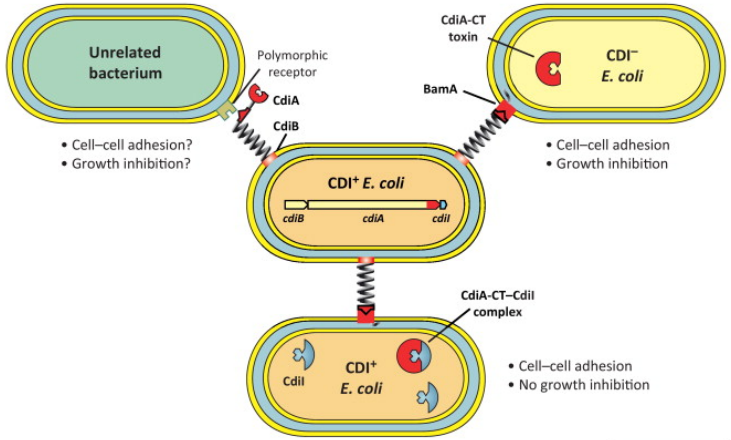
Julia Webb

Following CdiA transfer to the target cell surface:

- The CdiA-CT enters target cells.
- The CdiA N-terminus remains protease-sensitive

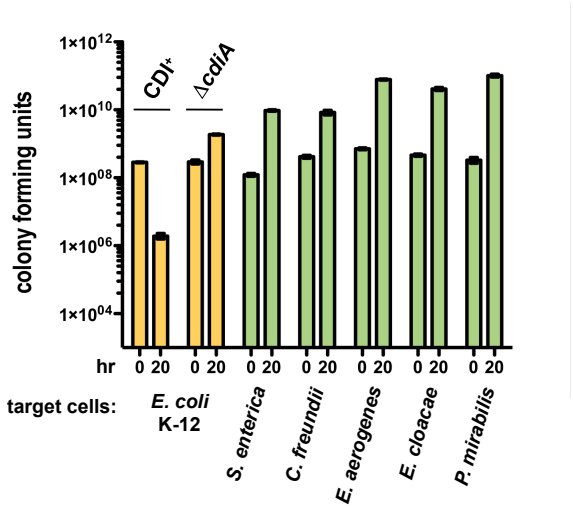


Self/Non-Self Discrimination in CDI systems



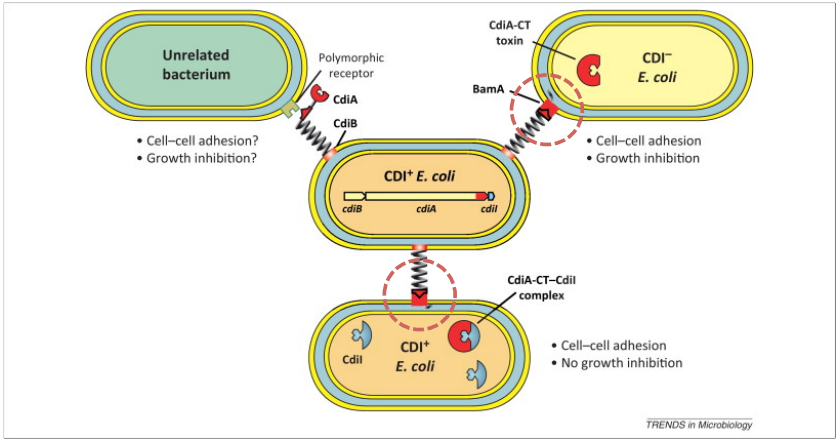
overview

E. coli EC93 versus related γ-proteobacteria



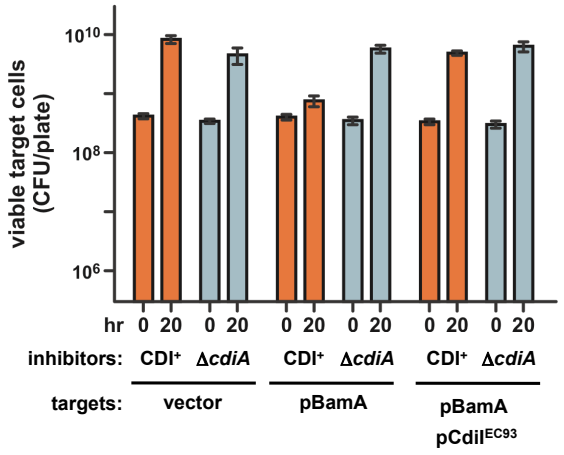
Zach Ruhe

BamA is the receptor for CdiA^{EC93}



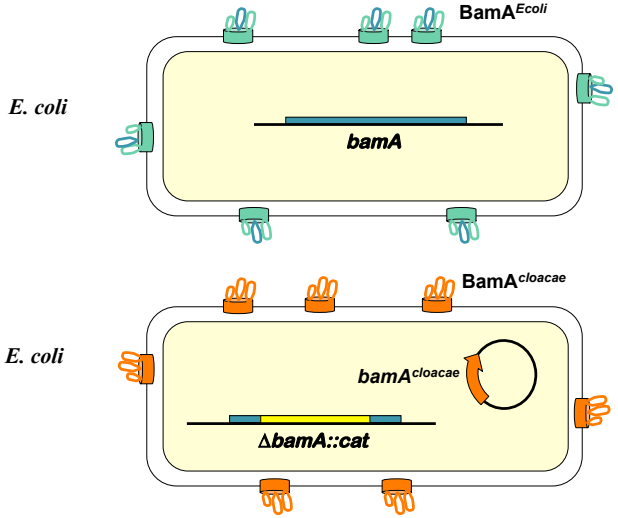
overview

E. coli BamA sensitizes *E. aerogenes* to CDI



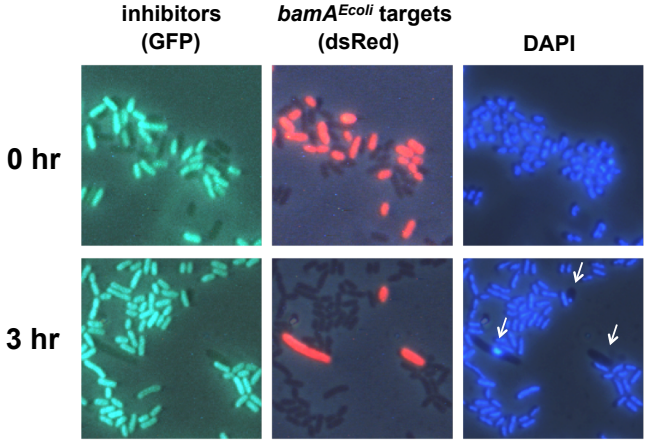
Zach Ruhe

Contact-dependent growth inhibition (CDI)



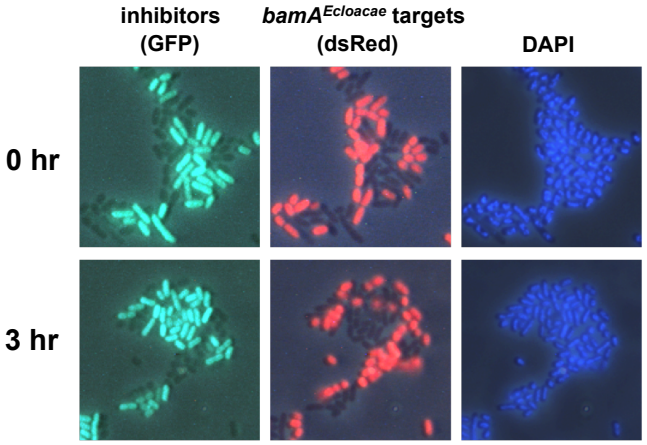
BamA from *E. cloacae is functional in E. coli*

Heterologous BamA provides resistance to CDI



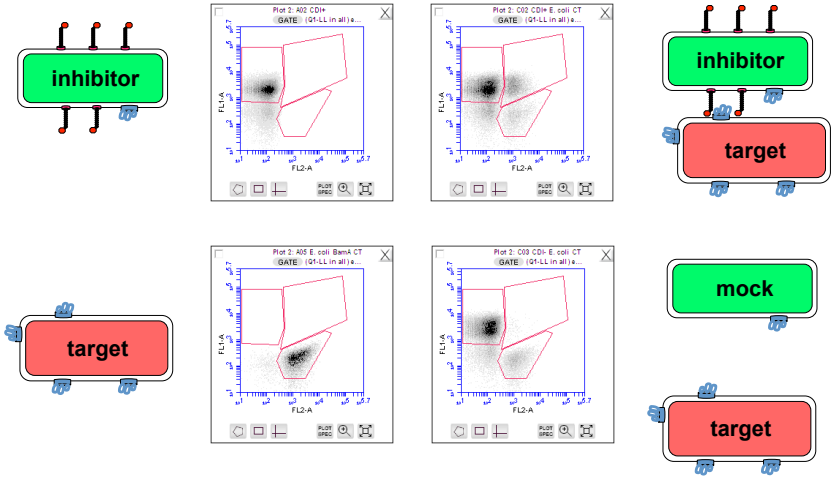
Kiel Nikolakakis

Heterologous BamA provides resistance to CDI

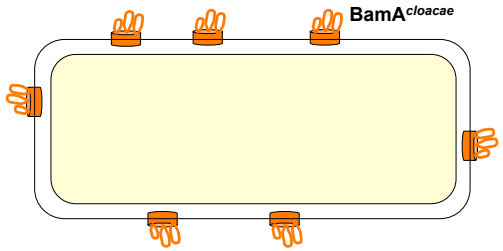


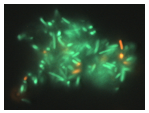
Kiel Nikolakakis

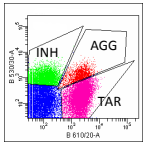
Cell-cell aggregation by flow cytometry



Heterologous BamA confers CDI-resistance to *E.coli*



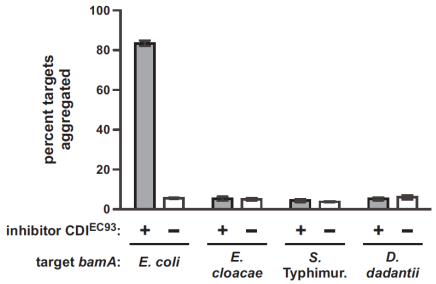




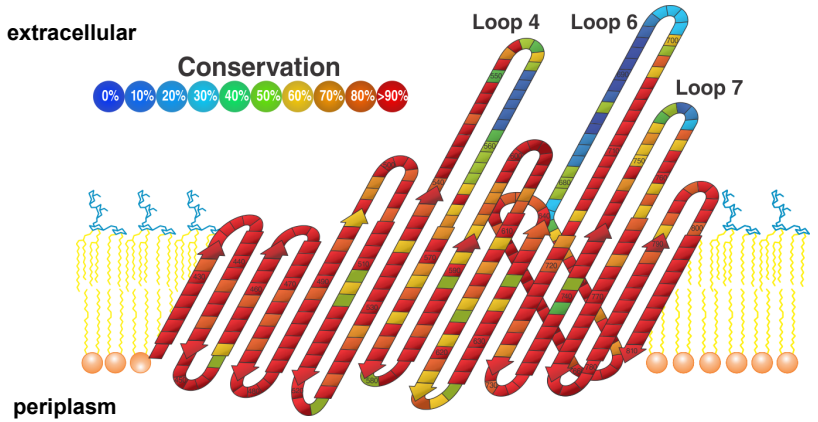
Red/Green labeling of targets & inhibitors

Flow cytometry:

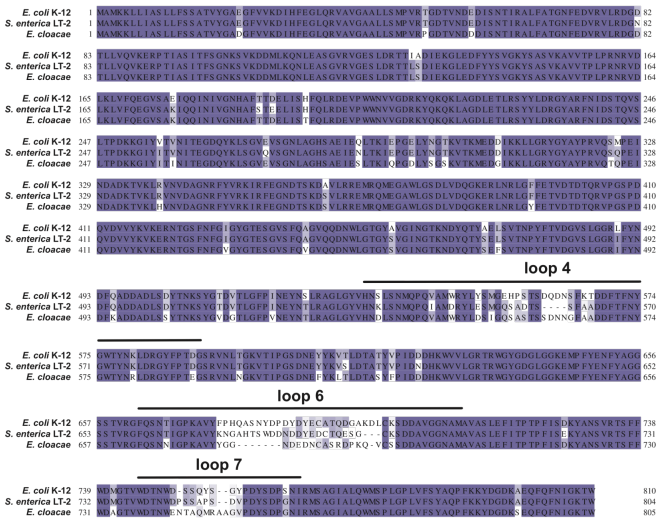
Inhibitor (INH)
Target (TAR)
Aggregates (AGG)



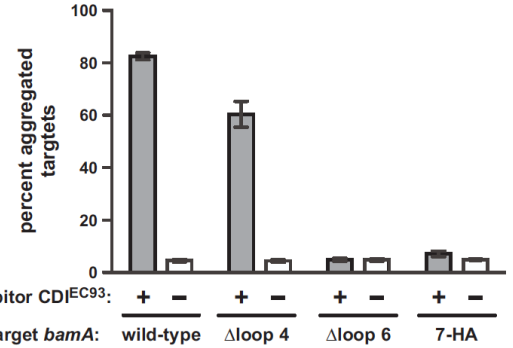
BamA conservation between γ-proteobacteria



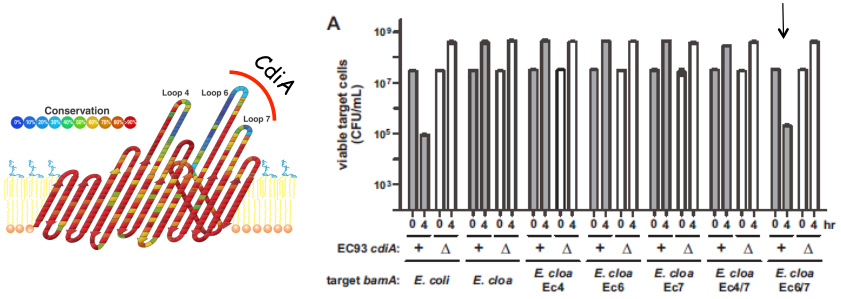
The extracellular loops of BamA are variable



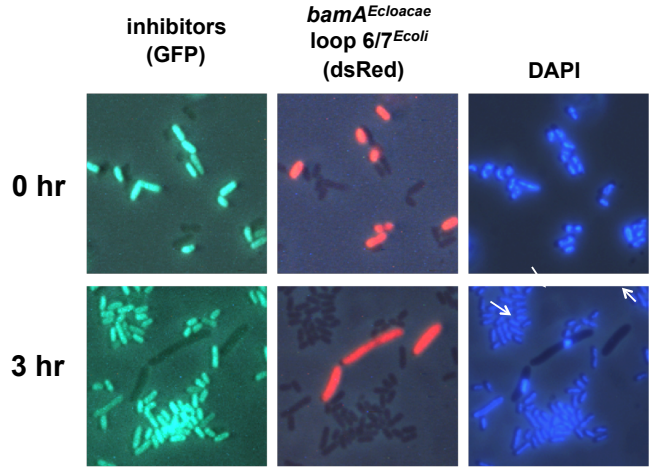
Evidence for role of BamA loops 6/7 in binding: Deletion Analysis



Evidence for role of BamA loops 6/7 in binding: Chimera Analysis



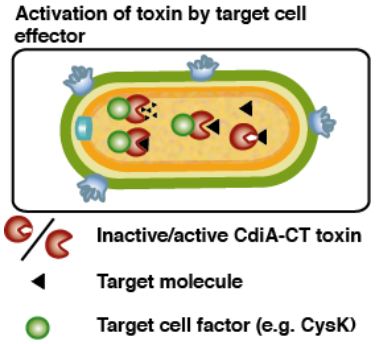
Key role for BamA loops 6/7 in species specificity



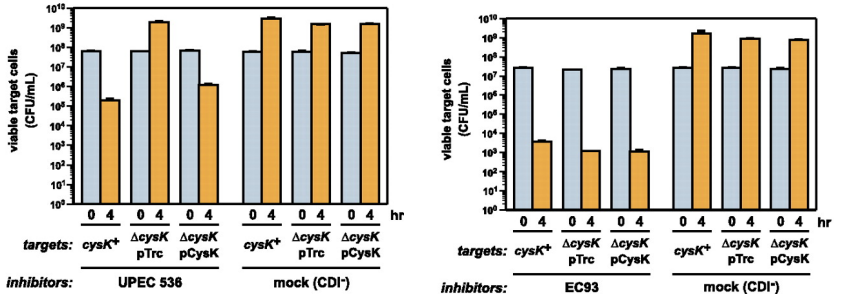
Kiel Nikolakakis

Some CDI toxins require activation by a host-cell factor

- Some CDI toxins are not active on their own
- Require a host-cell factor for activation – a permissive factor

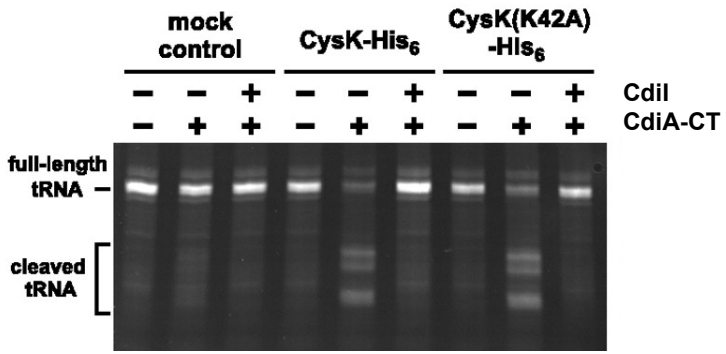


CysK is required for growth inhibition during CDI mediated by CdiA^{UPEC536}



Diner E J et al. Genes Dev. 2012;26:515-525

CysK is necessary and sufficient for tRNase activity in vitro



Diner E J et al. Genes Dev. 2012;26:515-525

The discovery of another CDI system

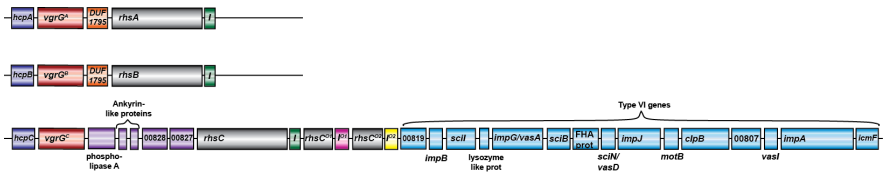
- RHS-proteins are used for CDI
  - Recombination Hot-Spots
  - Discovered for 40 years ago in *E.coli* K12
  - Found in the majority of all Gram-negative bacteria
  - Homologes found also in Gram-positive bacteria and in eukaryotes in the form of teneurins
  - Are used for CDI in:
    - *Salmonella typhimurium* LT2
    - *Pseudomonas aeruginosa*
    - *Dickeya dadantii*
    - *Enterobacter cloacae*
    - *Bacillus subtilis*

The discovery of another CDI system

- Rhs-proteins are modular with a highly diverse C-terminal domain and a conserved N-terminal "core" domain
- C-terminal domain contain the toxic activity and is delivered to target cells

Rhs-mediated CDI in *Dickeya dadantii*

- Plant pathogen
  - Infects potatoes, chicory, viola
  - Causes potato rot
- 3 Rhs-loci

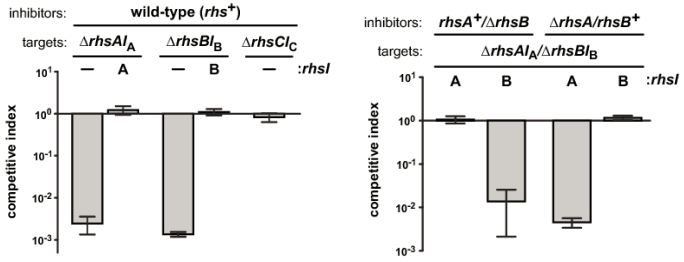


Competitive index = $\frac{\text{Ratio of targets / inhibitors at time 24h}}{\text{Ratio of targets / inhibitors at time 0h}}$

Koskiniemi et al. PNAS, 2013

Rhs-mediated CDI in *Dickeya dadantii*

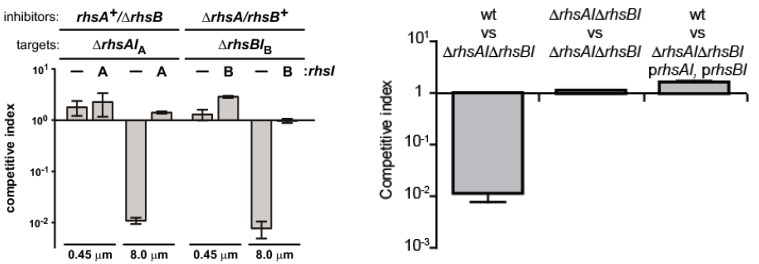
- 3 Rhs-loci
  - 2 are active in laboratory media (pH~7.3, 2% glucose)
  - Immunities are specific and protect against their cognate toxin



Koskiniemi et al. PNAS, 2013

Rhs-mediated CDI in *Dickeya dadantii*

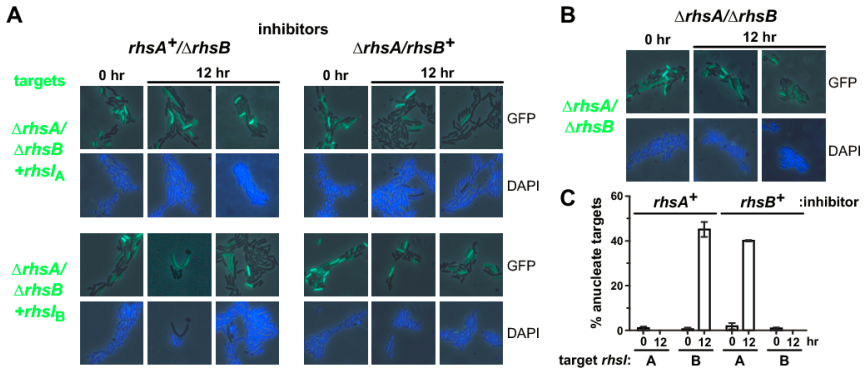
- 3 Rhs-loci
  - Inhibition is contact-dependent
  - Inhibition also in natural environment - potato



Koskiniemi et al. PNAS, 2013

Rhs-mediated CDI in *Dickeya dadantii*

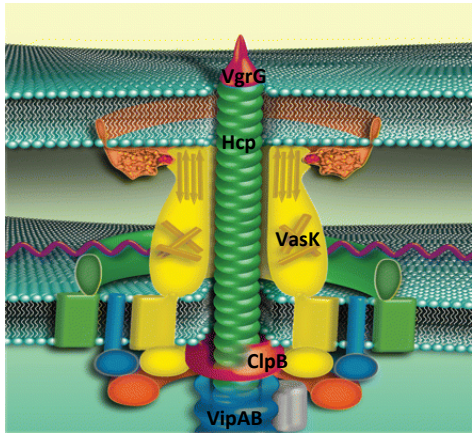
- RhsA and RhsB CT's encode DNAses that are delivered to target cells



Koskiniemi et al. PNAS, 2013

Rhs-mediated CDI in *Dickeya dadantii*

- Rhs-mediated inhibition requires components of the typeVI secretion system



Rhs-mediated CDI in *Dickeya dadantii*

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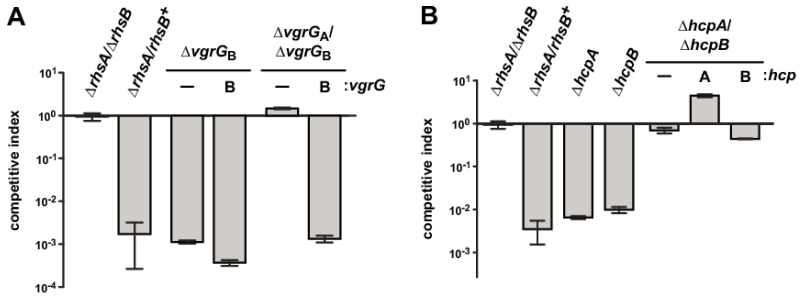
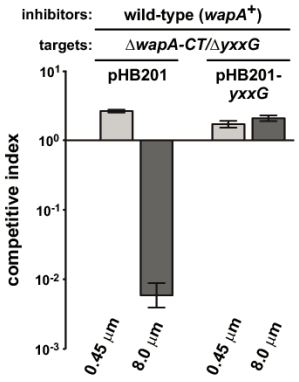


Koskiniemi et al. PNAS, 2013

Rhs-mediated CDI in *Bacillus subtilis*

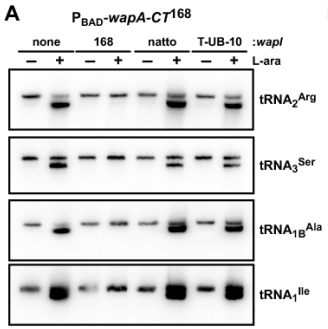
- Rhs homologue WapA mediates CDI
- Protected by cognate immunity
- No typeVI secretion system
  - WapA secreted by the general secretory pathway



Koskiniemi et al. PNAS, 2013

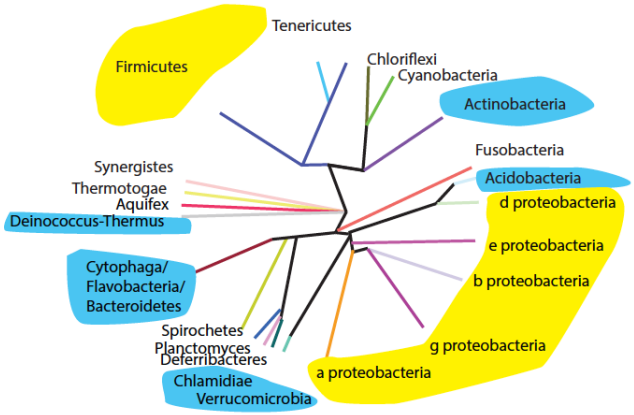
Rhs-mediated CDI in *Bacillus subtilis*

- WapA-CT from *B.subtilis* 168 has tRNAse activity
- Protected by cognate immunity



Koskiniemi et al. PNAS, 2013

CDI systems are everywhere



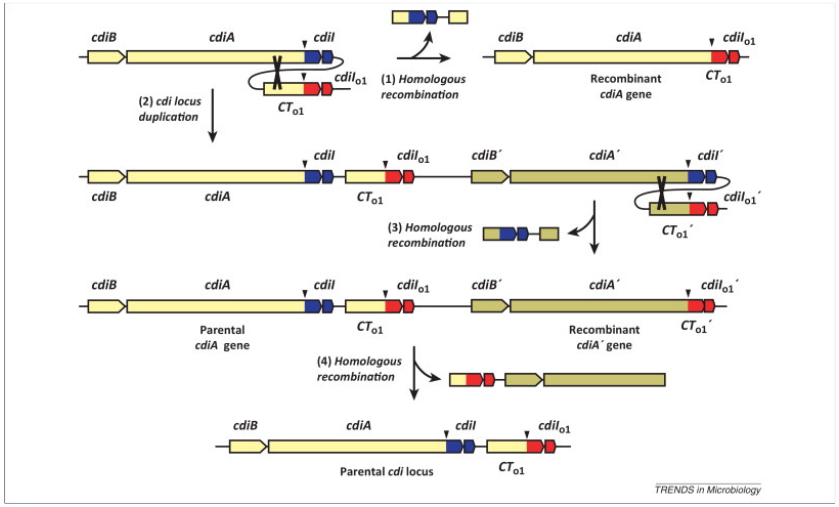
CdiA and Rhs

Rhs

Orphan Toxins – an arsenal of weapons?

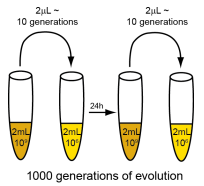
- *cdi* and *rhs* loci often contain additional toxin-immunity pairs that are not associated with a "stick" – so called orphan toxins
- These orphan toxins often lack translation initiation signals and it is unknown if they are expressed
- Orphan toxins from one strain can be found on full-length CdiA /Rhs sticks in other strains

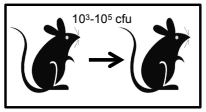
Orphan Toxins – an arsenal of weapons?



Rhs-mediated CDI in *Salmonella typhimurium* LT2

- Our model organism: *Salmonella enterica* serovar Typhimurium LT2 (*S.typhimurium LT2*)
- Serial passage for faster growth in laboratory media (LB-broth)
  - 1000 generations
- Serial passage for increased virulence in mice (i.p.)
  - 150 generations





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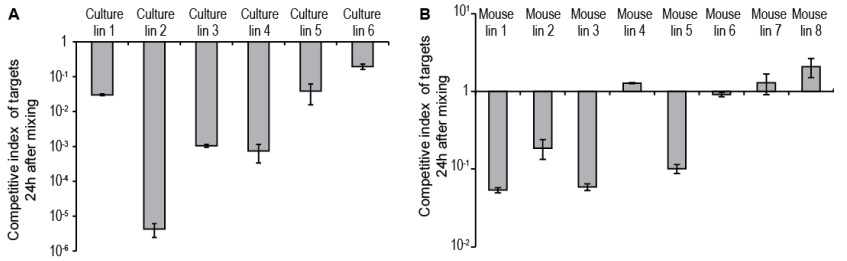
Evolved lineages outcompete parental wild type strain



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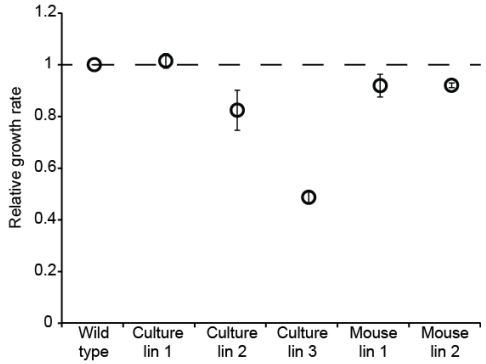
Evolved lineages grow slower than the parental wild type strain



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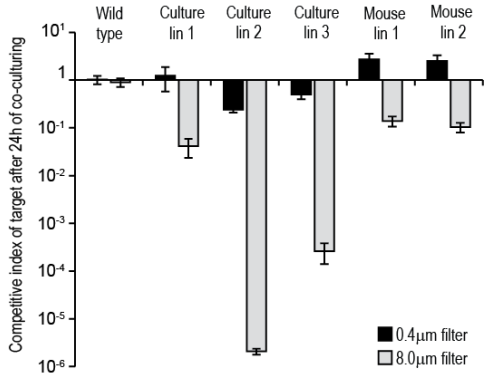
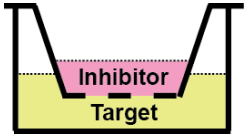
Inhibition requires direct cell-to-cell contact





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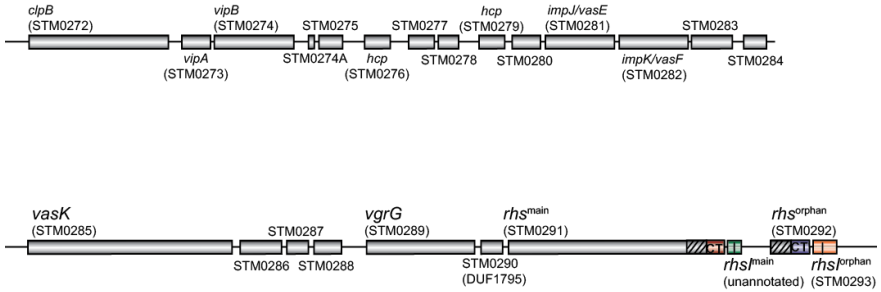
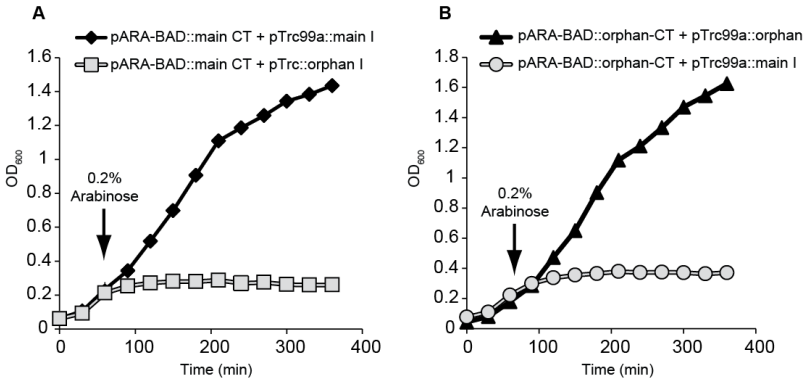
Rhs-mediated CDI in *Salmonella typhimurium* LT2

- 1 *rhs*-loci
- 1 orphan toxin



Rhs-toxins in *S.typhimurium*



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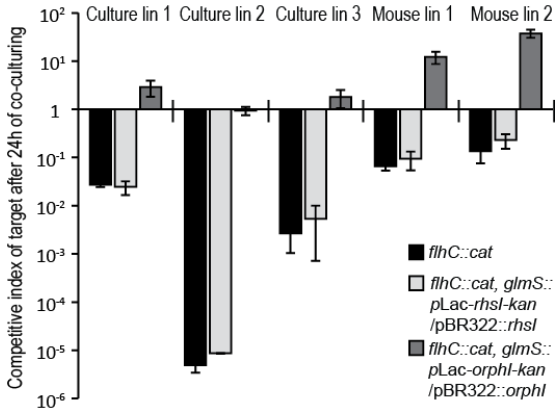
Rhs-orphan immunity protects parental strain from inhibition from the evolved populations



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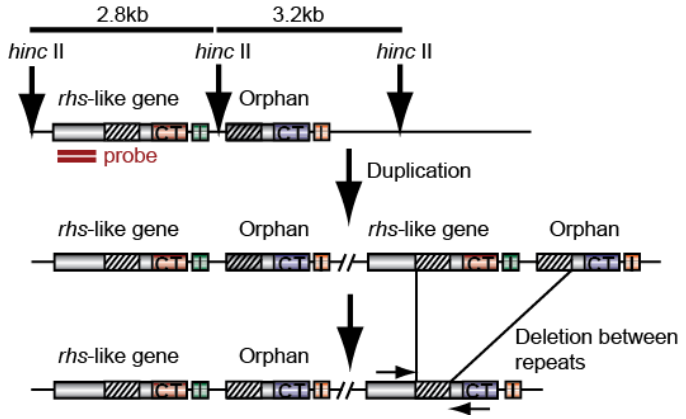
Model



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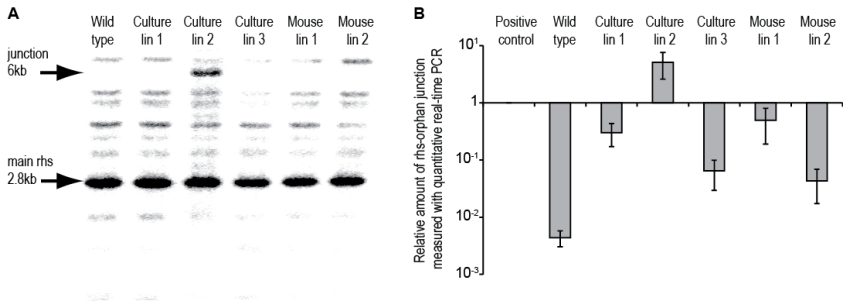
Duplication and a subsequent deletion results in expression of a new orphan-toxin on the main Rhs-stick



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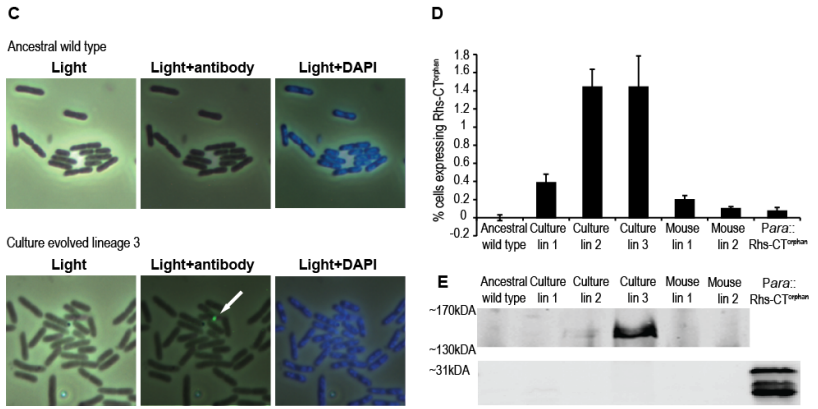
Expression of Rhs orphan-CT on cell surface



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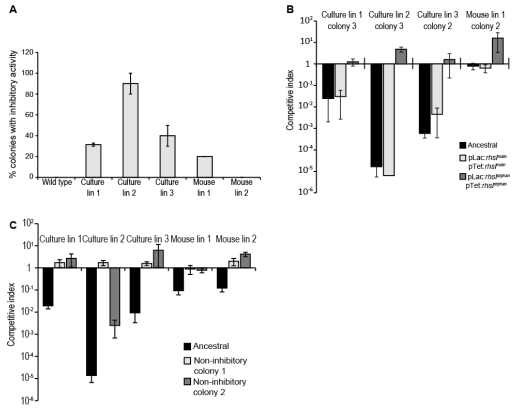
Single colonies from evolved cultures inhibit parental strain



Conclusions / things for discussion

- Systems for contact-dependent growth inhibition can be found throughout the bacterial kingdom
- Rhs proteins are some of the most highly positively selected genes known
- Bacteria with these systems are able to stop the growth of other bacteria of the same species but not bacteria from other species
- Orphan toxins represent a silent arsenal of weapons that can be mobilized for clonal selection

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