



## Commensal *E. coli* enhance toxin production by *Escherichia coli* O157:H7

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### Penn State E. coli Reference Center (Dept. Food Science)

- Established in 1967, Dr. Paul Glanz
- >89,000 isolates collected over 50 years
  - 353 species or origins
  - 282 countries or regions
  - >4,000 genome sequences
- Diagnostic tests: serotyping, PCR panels
- 2015, 2017 International *E. coli* molecular serotyping conference
- https://foodscience.psu.edu/research/centers/ecoli
- @PSU\_EcoliRefCtr





## Escherichia coli O157:H7

- Serotype
  - O: somatic antigen
  - H: flagella antigen
- Cattle are the main reservoir
- US CDC: 63,000 cases per year
- Low infectious dose
- Causes severe diseases
  - Hemorrhagic colitis (HC)
  - Hemolytic uremic syndrome (HUS)

Ingestion

Diarrhoea

Abdominal

pain

Fever

Vomiting

Culture

Bloody

diarrhoea (~90%)





Spontaneous

resolution

(~85%)

## Major virulence factors of O157:H7

- Shiga toxin
  - Two subunit (AB<sub>5</sub>)
  - Two types: Stx1 and Stx2



- Allelic variants: Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, Stx2g, Stx2h, Stx2i
- Locus of enterocyte effacement
  - Intimin (EaeA) and Tir
  - Type III secretion effectors



## Shiga-toxin converting bacteriophage







Co-culture

### Supernatant assay



Toxin quantification by ELISA

SOS inducible *gfp* reporter strain

# PA2 is the highest toxin producer when co-cultured with *E. coli* C600



## **Co-infection increases disease severity**

<u>*</u>			
E. coli inoculum	No. healthy	No. sick	No. moribund/dead
PA2 only	$11^a$	0	0
PA2 + C600	2	5	3

<sup>*a*</sup> The PA2-plus-C600-infected mice had a higher morbidity and mortality rate than mice infected with PA2 alone (P < 0.05 by chi-square test).





Coculture of *Escherichia coli* O157:H7 with a Nonpathogenic *E. coli* Strain Increases Toxin Production and Virulence in a Germfree Mouse Model

Kakolie Goswami,<sup>\*\*</sup> Chun Chen,<sup>\*\*</sup> Lingzi Xiaoli,<sup>\*</sup> Kathryn A. Eaton,<sup>\*</sup> Edward G. Dudley<sup>\*,b</sup> Department of Food Science<sup>\*</sup> and Center for Immunology and Infectious Disease<sup>\*</sup>, <sup>p</sup>erno State University, University Park, Pennsylvania, USA; Department of Microbiology and Immunology, University of Microbiology. Johnson 2004







\*not to scale

# *E. coli* 1.1954 mediated amplification of Stx2a amplification is *bamA*-independent



"EE" strains express E. coli BamA

"K2" strains express Salmonella BamA

Non-pathogenic *Escherichia coli* Enhance Stx2a Production of *E. coli* O157:H7 Through Both *bamA*-Dependent and Independent Mechanisms

Lingzi Xiaoli  $^1\!,$  Hillary M. Figler  $^2\!,$  Kakolie Goswami Banerjee  $^1\!,$  Christopher S. Hayes  $^3$  and Edward G. Dudley  $^{1.4*}$ 





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New Results

Cold

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#### A putative microcin amplifies Shiga toxin 2a production of Escherichia coli O157:H7

Hillary M. Figler, Lingzi Xiaoli, Kakolie Banerjee, Maria Hoffmann, Kuan Yao, Edward G. Dudley doi: https://doi.org/10.1101/646182

### Other non-pathogenic E. coli increase Stx2a level



### Strain 0.1229 amplifies Stx2a and induces SOS response



One-way ANOVA Dunnett's test, p<0.05 Compared to LB

## Stx2a amplification by 0.1229 is sensitive to Proteinase K



Two-way ANOVA Dunnett's test, p<0.05 Compared to untreated

# CFT073 and Nissle do not promote Stx2a amplification









One-way ANOVA Dunnett's test, p<0.05 Compared to LB





One-way ANOVA Dunnett's test, p<0.05 Compared to LB



# Activity is encoded within 5.2 kb of p0.1229\_3

80-

60-

40-

20-

0

\*

\*

\*

Stx2a (µg/ml/OD)



LB

One-way ANOVA Dunnett's test, p<0.05 Compared to 0.1229

### At least four ORFs are essential for function



One-way ANOVA Fisher's LSD test, p<0.05 Compared to 0.1229

### Microcins



### Some microcins use TolC for export



### Putative microcin in 0.1229 requires tolC



One-way ANOVA Dunnett's test, p<0.05 Compared to LB

### Some microcins use TonB for entry into target cell



### Putative microcin in 0.1229 requires tonB



Two-way ANOVA Dunnett's test, p<0.05 Compared to each LB



\*not to scale

# Are there other *E. coli* with similar Stx2a amplification mechanisms?



### Three out of 101 human fecal isolates amplify GFP



### Two isolates encode the same microcin as 0.1229



### Other bacterial species may encode the microcin





### *E. coli* strain 0.1229

- Amplifies Stx2a of O157:H7
- Secretes putative microcin into supernatant
- Encodes three plasmids
  - Genes necessary for amplification are found on p0.1229\_3
- At least four ORFs on p0.1229\_3 are required for Stx2a amplification
  - Hp2 is not part of the conserved unit
- 5.2kb region is sufficient for amplification
- Requires *tolC* for export from producing cell
- Requires tonB for entry into target cell





Does the presence of 0.1229 (or similar *E. coli*) make an individual at higher risk for HUS?

Can we use this information to develop better treatment options?







National Institute of Allergy and Infectious Diseases



PennState Huck Institutes of College of Agricultural Sciences

**PennState** 

3

Food and Agricultural Sciences National Needs Graduate Fellowship

Graduate Research Innovation Grant





Maria Hoffmann (FDA CFSAN) Roberto Kolter (Harvard Medical School) Craig Stephens (Santa Clara University)