National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

STEC in the environment and plants

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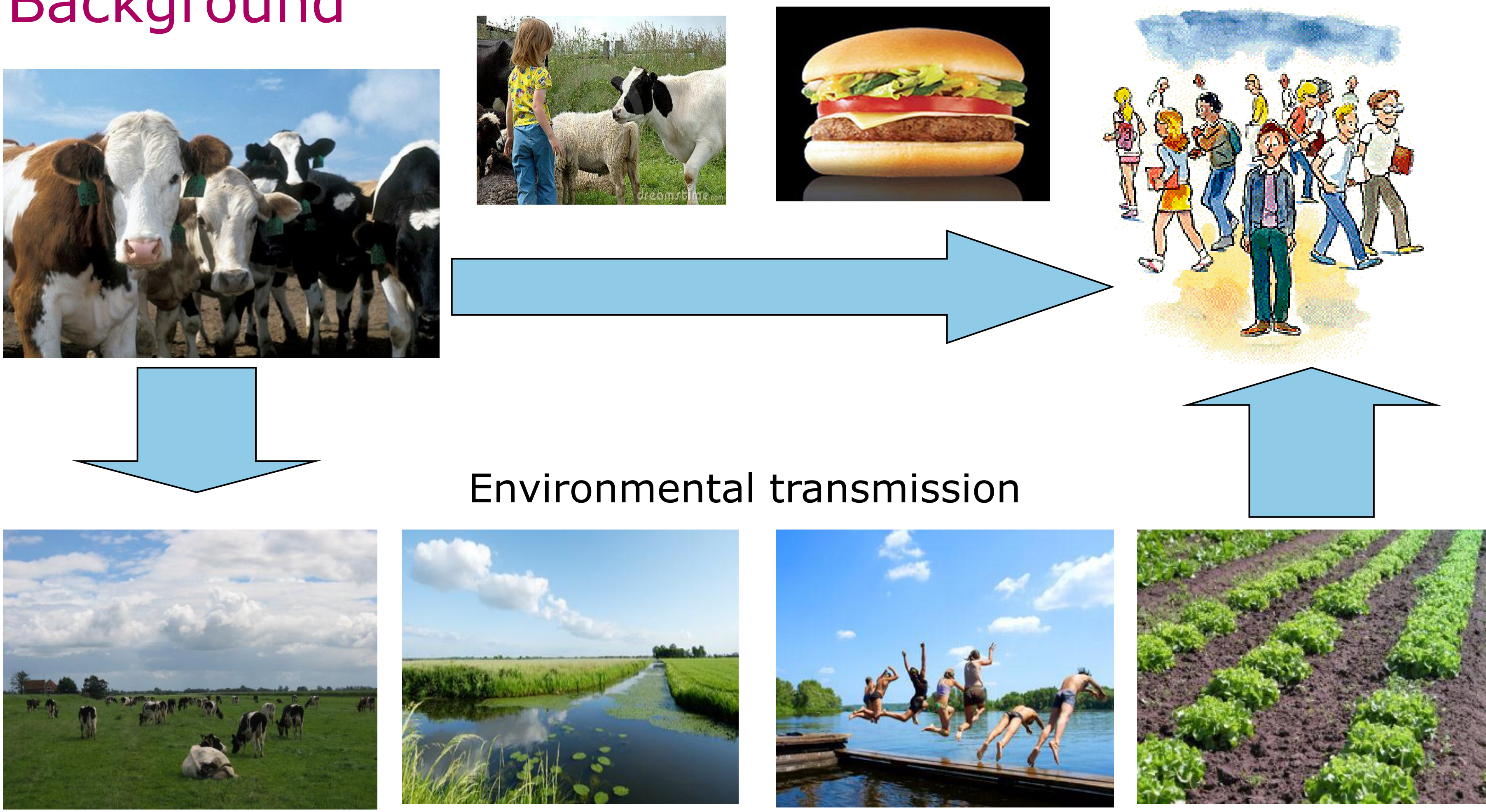
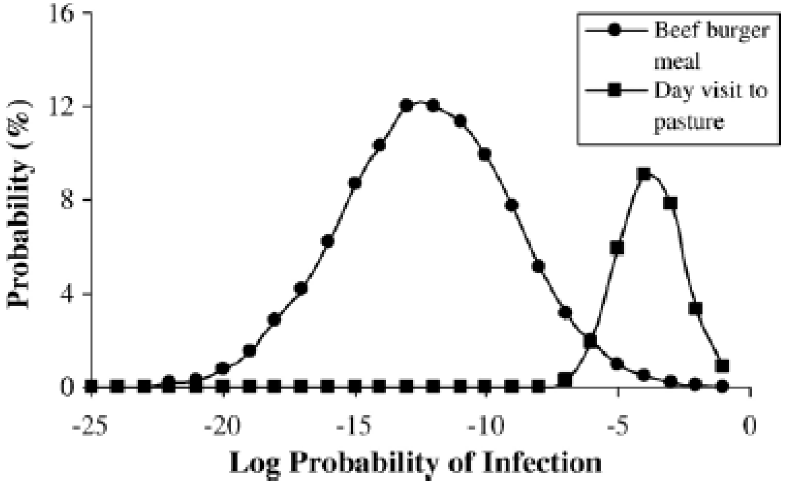
Background

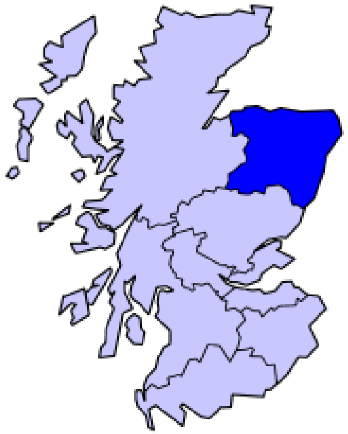


Importance environmental transmission

Strachan 2006 Int. J. Food Microb. 112

Higher risk visiting pasture
compared to eating burger





Ingestion of fecal and/or soil material?

Increased STEC transmission by fresh vegetables?

Jaar	Land	Pathogeen	Product	#cases
1996	Japan	*E. coli* O157	Radish sprouts	> 10,000
2005	Sweden	*E. coli* O157	Lettuce	135
2006	U.S.	*E. coli* O157	Spinach	238
2007	Netherlands	*E. coli* O157	Lettuce	35
2008	U.S.	*E. coli* O157	Lettuce	74
2010	U.S.	*E. coli O*145	Lettuce	33
2011	Germany	*E. coli* O104:H4	Fenegreek sprouts	3842 (HUS:855/†53)
2012	U.S.	*E. coli* O26	Clover sprouts	29
2012	Japan	*E. coli* O157	Pickled cabbage	110 (†7)?

Ongoing O157 outbreak U.S. spinach

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NY Spinach E. coli Outbreak Rises to 19

BY **NEWS DESK** | NOVEMBER 5, 2012

At least 19 New Yorkers have fallen ill in an E. coli O157:H7 outbreak linked to spinach salad mix purchased at Wegmans stores. That number has risen from the 16 cases first confirmed by the New York State Department of Health on Friday.

Of those ill, five have been hospitalized, with four already having been discharged.

The illnesses are spread across five counties: Monroe, Niagara, Steuben, Wayne and Erie.

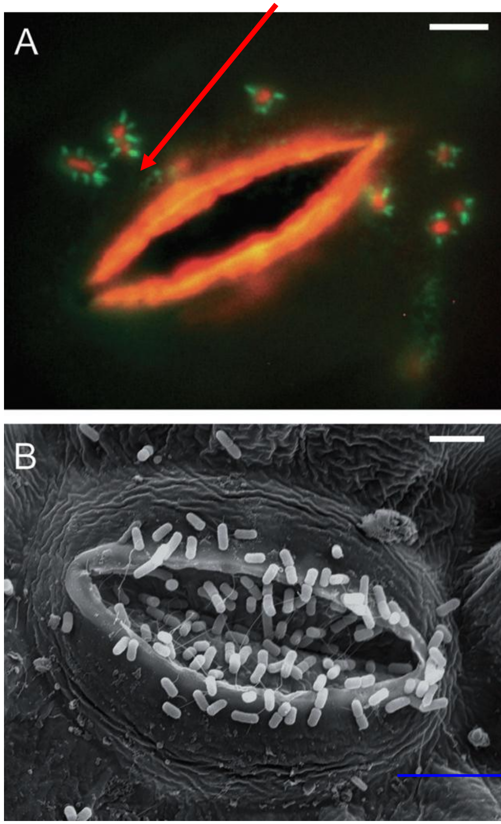
Wegmans issued a voluntary recall of their 'Wegmans Food You Feel Good About Organic Spinach & Spring Mix' on Thursday due to possible E. coli contamination.

Food safety concerns fresh vegetables

- Low VTEC prevalence but large consumption amounts
- Raw consumption
- Limited efficiency of washing and/or decontamination
  - Strong surface attachment (Jeter 2005, Shaw 2008, Xicohtencatl 2009)
  - Presence in deeper plant tissue/vascular system (Solomon 2007, Franz 2007)
- Potential for large-scale spread when "fresh-cut"
- Vulnerable for **environmental contamination** when grown in the field
  - Soil, manure as fertilizer, irrigation water, wildlife
- Potential for large-scale spread when "fresh-cut"
- Abundance of simple sugars in/on (damaged) leaves

Attachment *E. coli* O157 to leaves

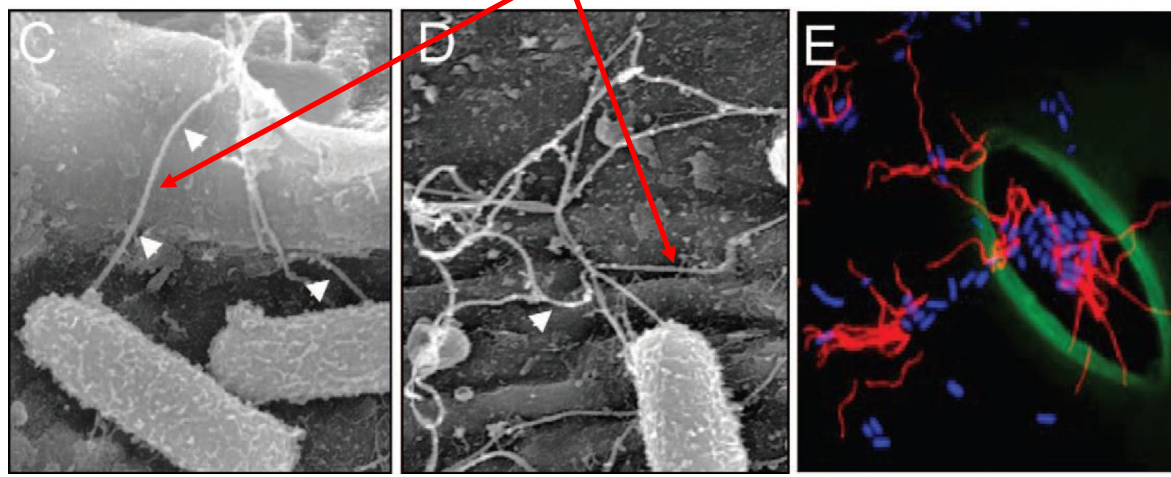
T3SS filaments



Shaw 2008

Flagella

Xicohtencatl 2009



Also curli and cellulose (Macarisin 2012)

Attachment processes similar to epthelial cells

→ Protective, nutrient rich locations

The life-style of *E. coli* is biphasic

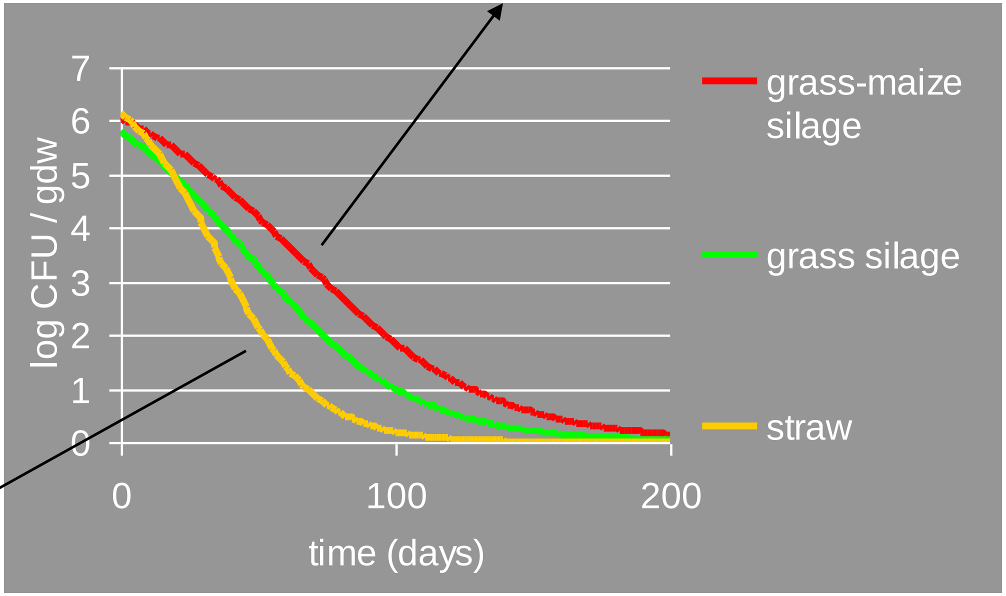
Host-dependent phase	**Host-independent phase**
Host /reservoir	**Environment / Food**
Livestock Human Wildlife	Manure Water Soil Plants
High nutrient availability Optimal temperature Anaerobic	Nutrient limitation Low temperatures High UV, low Aw Aerobic
STABLE	FLUCTUATING
NET GROWTH	NET DECLINE

E. coli is adapted to fast growth (copiotrophic) on mainly simple sugars

Survival in manure: nutrient status

E. coli O157:H7

Low fibre content:
high level simple sugars
High pH

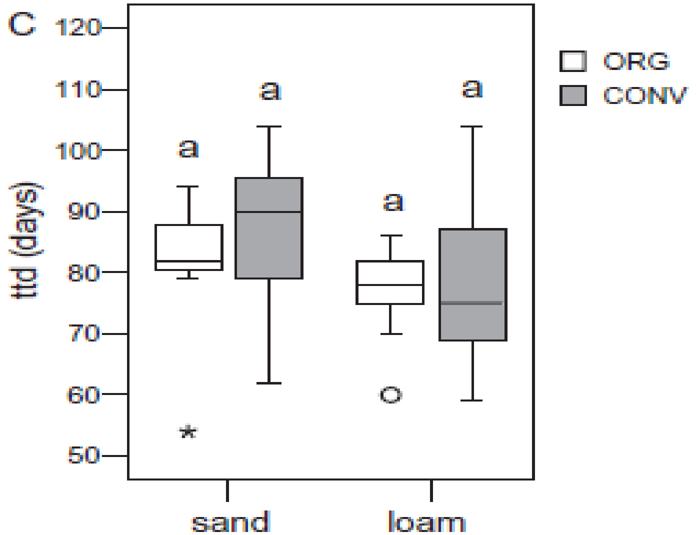


High fibre content:
Low level simple sugars: O157 depended on degradation complex sugars by other bacteria/fungi
High pH

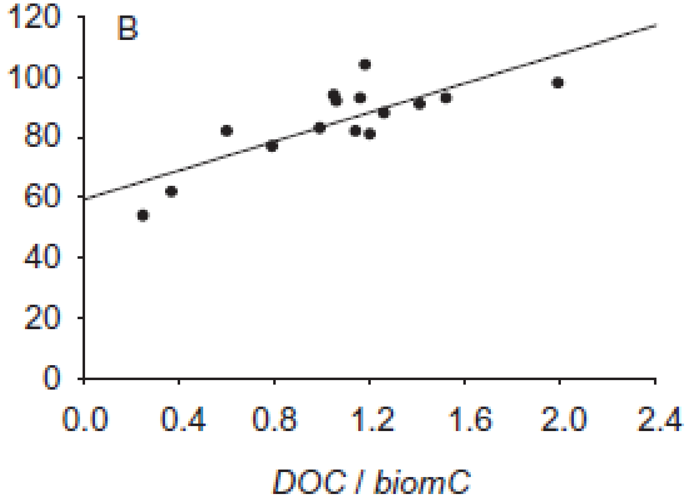
Survival in soil: nutrient status

E. coli O157:H7 survival in 36 different soils

No differences management and soil type



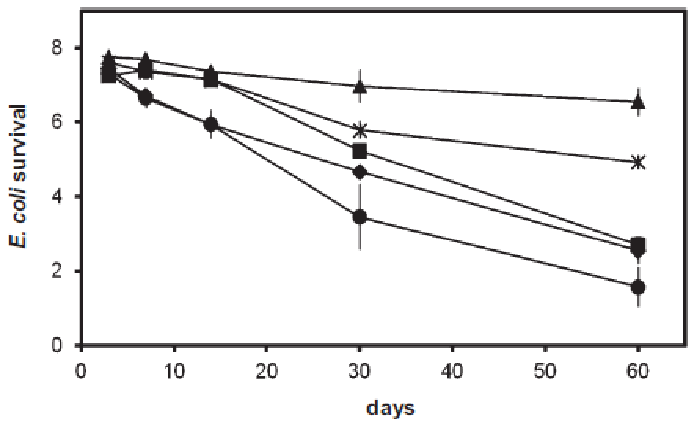
Strong relation with level dissolved simple sugars per unit biomass carbon



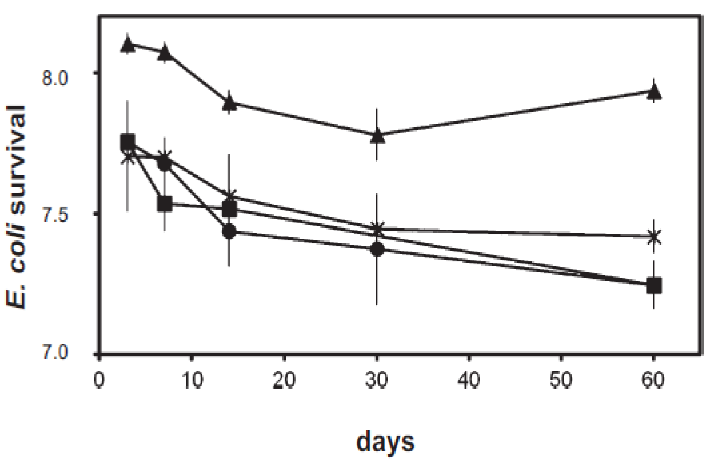
Van Elsas 2012 PNAS 109:4

Survival soil: microbial species diversity

O157 survival in soil with artificially altered microbial diversity



Increased dilution

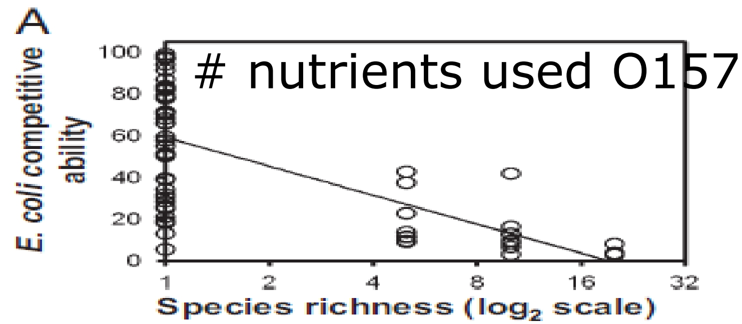


Sterile

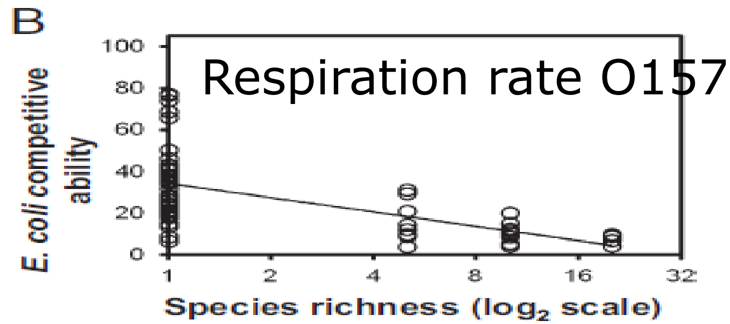
5 strains

20 strains

100 strains



nutrients used O157



Respiration rate O157

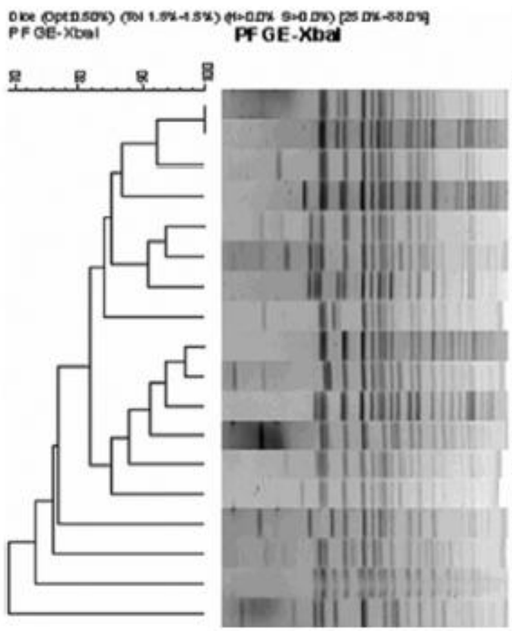
Higher microbial diversity in soil

Lower metabolic fitness O157

Survival in manure-amended soil: strain diversity

18 *E. coli* O157 strains in 1 manured soil

→ Large variation in survival: 47-266 days to detection limit
→ 7 Long survivors: 5 sheep, 1 food
→ No relation with PFGE, LSPA-lineage, clade



		Strain characteristics				Weibull model		
strain	source	O-type	H-type	LSPA lineage	clade 8	b	n	TTDL
m630	clinical human	O157	H-	I/II	-	2.47	0.52	119
m637	clinical human	O157	H7	I/II	-	5.58	0.55	215
m633	clinical human	O157	H-	I/II	-	2.41	0.43	266
m638	clinical human	O157	H7	I/II	-	5.09	0.53	228
m634	clinical human	O157	H7	I/II	-	5.39	0.64	104
m628	feces cow	O157	H7	I/II	+	2.36	0.49	121
m639	clinical human	O157	H7	I/II	-	2.82	0.45	242
m623	clinical human	O157	H-	I/II	-	2.83	0.5	155
m636	clinical human	O157	H7	I/II	-	8.18	0.86	71
m625	feces sheep	O157	H7	II	+	2.45	0.43	249
m635	clinical human	O157	H7	II	+	3.75	0.49	211
m631	feces cow	O157	H7	II	+	8.48	0.76	96
m622	raw-milk cheese	O157	H-	II	-	6.22	0.56	214
m619	feces lamb	O157	H-	II	+	7.13	0.83	71
m627	feces horse	O157	H7	I/II	+	4.68	0.75	68
m626	feces sheep	O157	H7	I/II	-	8.13	0.94	67
m632	feces cow	O157	H7	II	-	6.42	0.81	67
m629	feces cow	O157	H-	I	-	6.01	0.95	47

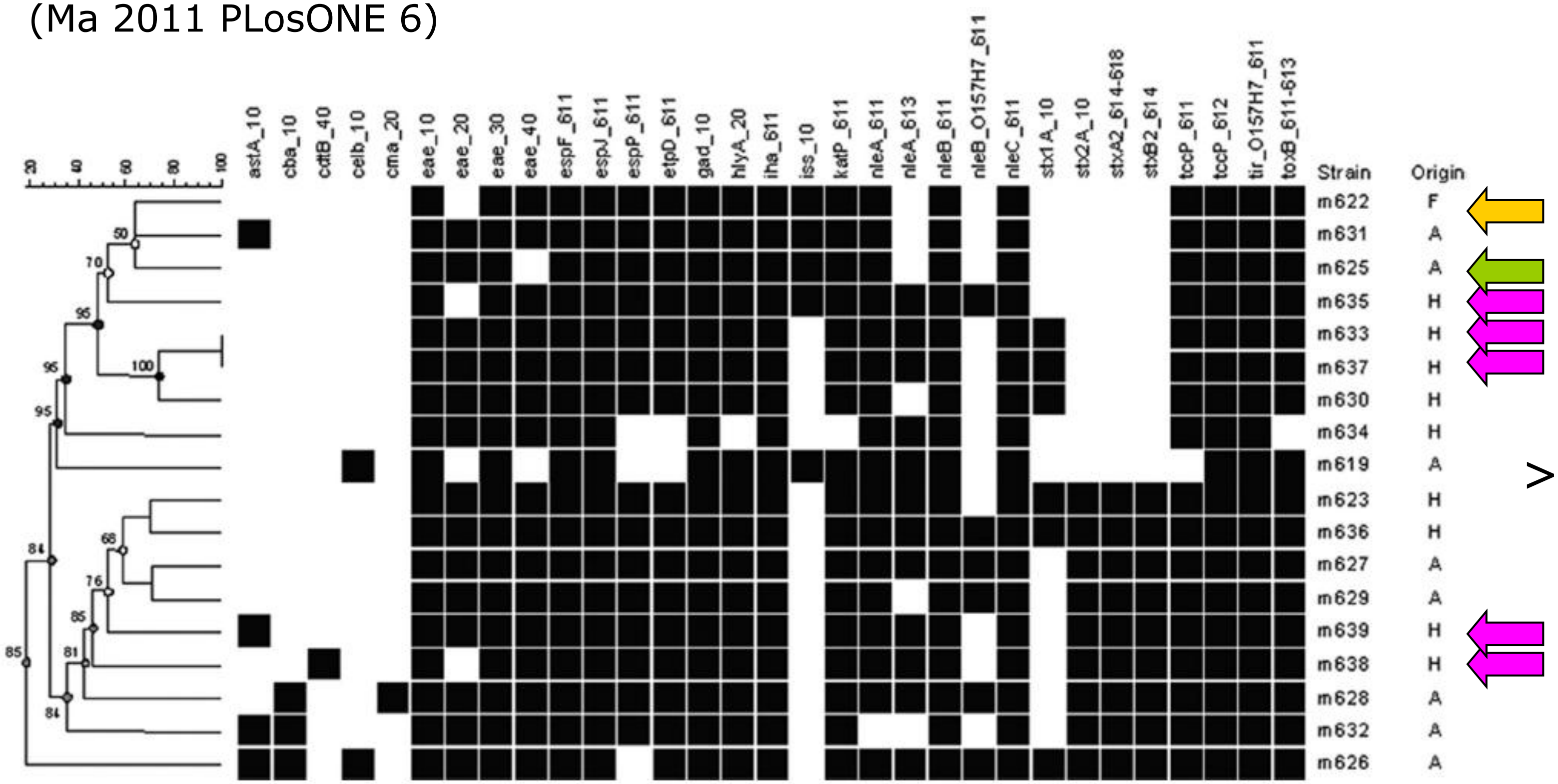
>200 days

Survival in manure-amended soil: virulence genes

→ No relation survival with virulence profile

→ Survival *E. coli* O157:H7 wildtype = Δ*stx1* = Δ*stx2* = Δ*eae* **=** Δ*stx1-2*

(Ma 2011 PLosONE 6)

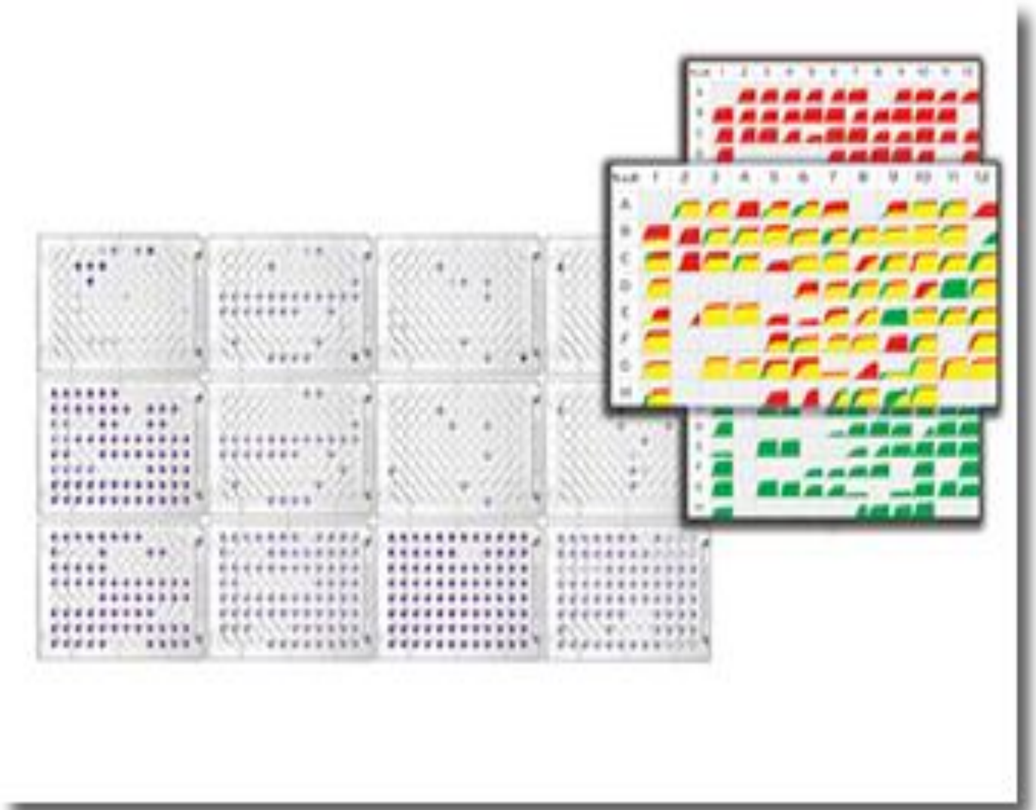


>200 days

Franz 2011 AEM 77:22

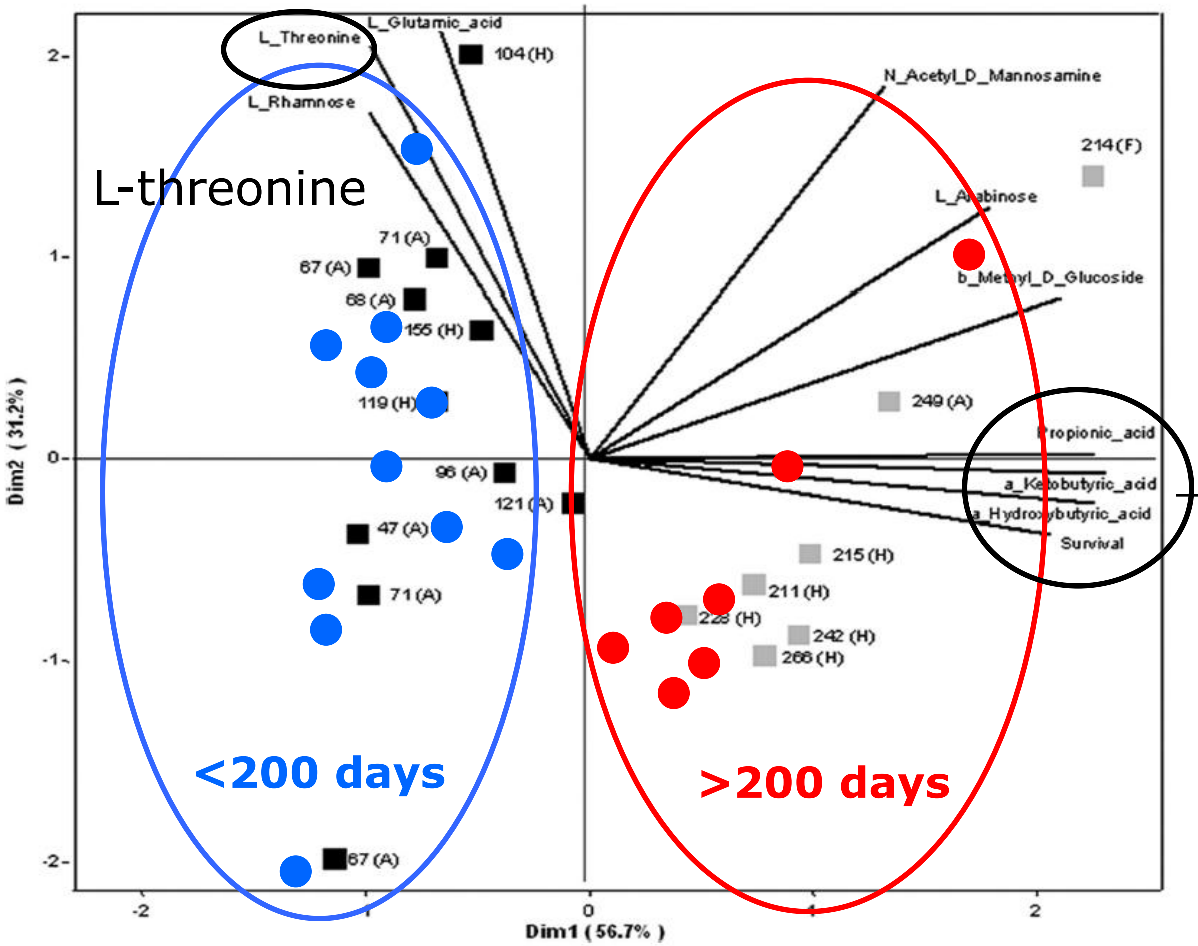
Survival in manure-amended soil: metabolism

Biolog metabolic profiling: substrate depending dye reduction



2 plates carbon sources
1 plate nitrogen sources
1 plate phosphorus sources
1 plate sulfur sources

Survival in manure-amended soil: metabolism



Propionic acid
a-ketobutyric acid
A-hydroxybutyric acid

Fatty acids in manure

Metabolic regulation by *rpoS*

rpoS: → Regulatory mechanism general stationary phase stress response
→ Positively regulates carbohydrate and fatty acid metabolism

rpoS WT versus Δ*rpoS*

Carbon source	PM-value
β-Methyl-D-Glucuronic Acid	102
L-Galactonic Acid-g-Lactone	98
L-Threonine	92
L-Alaninamide	70
L-Glutamine	67
L-Proline	66
D-Trehalose	64
D-Saccharic Acid	50
Propionic Acid	-51
Glycyl-L-Proline	-69
α-Keto-Butyric Acid	-86
α-Hydroxy-Butyric Acid	-110

Higher in Δ*rpoS*

Lower in *rpoS*$^{\mathbf{mut}}$

RpoS mutants: lower stress resistance, lower fatty acid metabolism
higher nutrient scavaging (broader “diet”; N-sources)

- Franz et al 2012, JCM
- Van Hoek, Franz, FEMS microb. Lett.

Survival in soil manure-amended: role of RpoS

Sequencing *rpoS* gene of all 18 *E. coli* O157 soil strains (Sakai template)

< 200 days survival

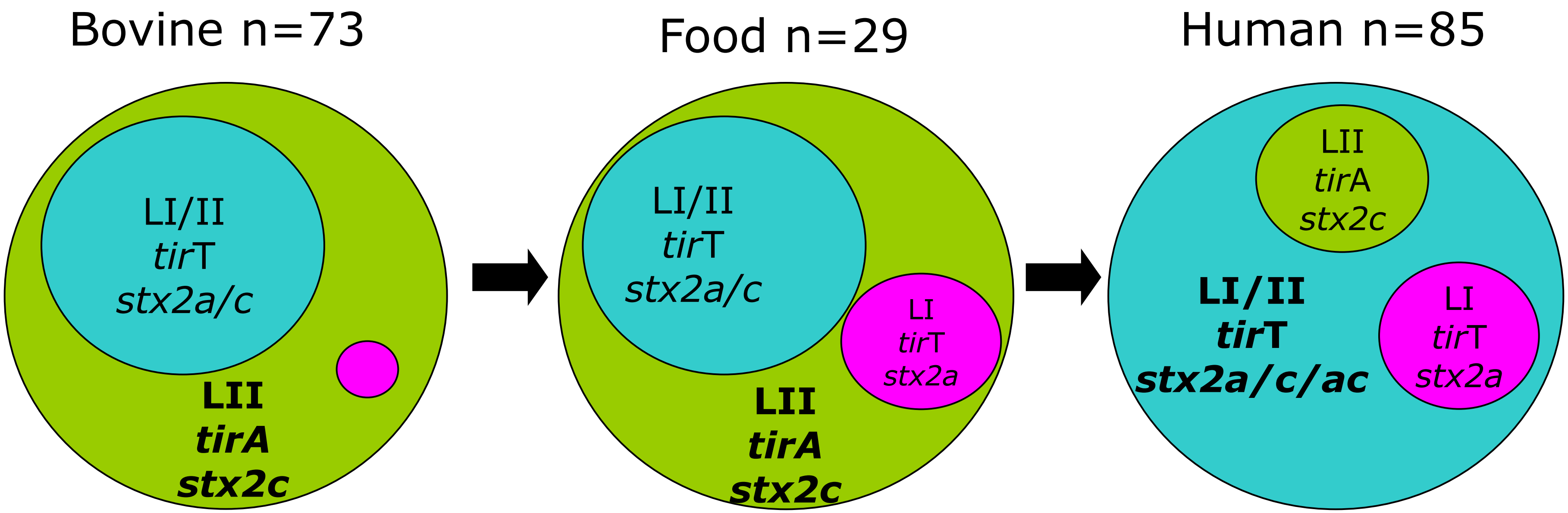
Strain	LSPA	*rpoS* mutation
M619	I	T433G (Y145D)
M623	I/II	T402G (F134L)
M626	I/II	488, insert 1bp
M627	I/II	396, insert 12bp
M628	I/II	T383A (I128N)
M629	I	654, deletion 13bp
M630	I/II	97, deletion 4bp
M631	II	75, deletion 1bp
M632	II	G376A (G126R)
M634	I/II	A917C (Q306P)
M636	I/II	C601T (Q201Stop)

> 200 days survival

Strain	LSPA	*rpoS* mutation
M622	II	-
M625	II	-
M633	I/II	-
M635	I/II	-
M637	I/II	-
M638	I/II	-
M639	I/II	-

Average survival pH 2.5 for 6h
63%±25 vs 97%±2 (P=0.003)

rpoS distribution among *E. coli* O157 isolates



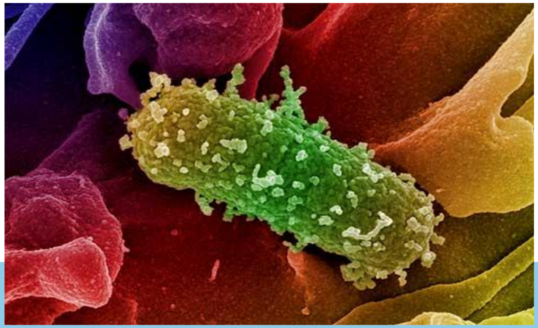
73 WT *rpoS*

27 WT *rpoS*
2 mutant *rpoS*

85 WT *rpoS*
28 mutant *rpoS*

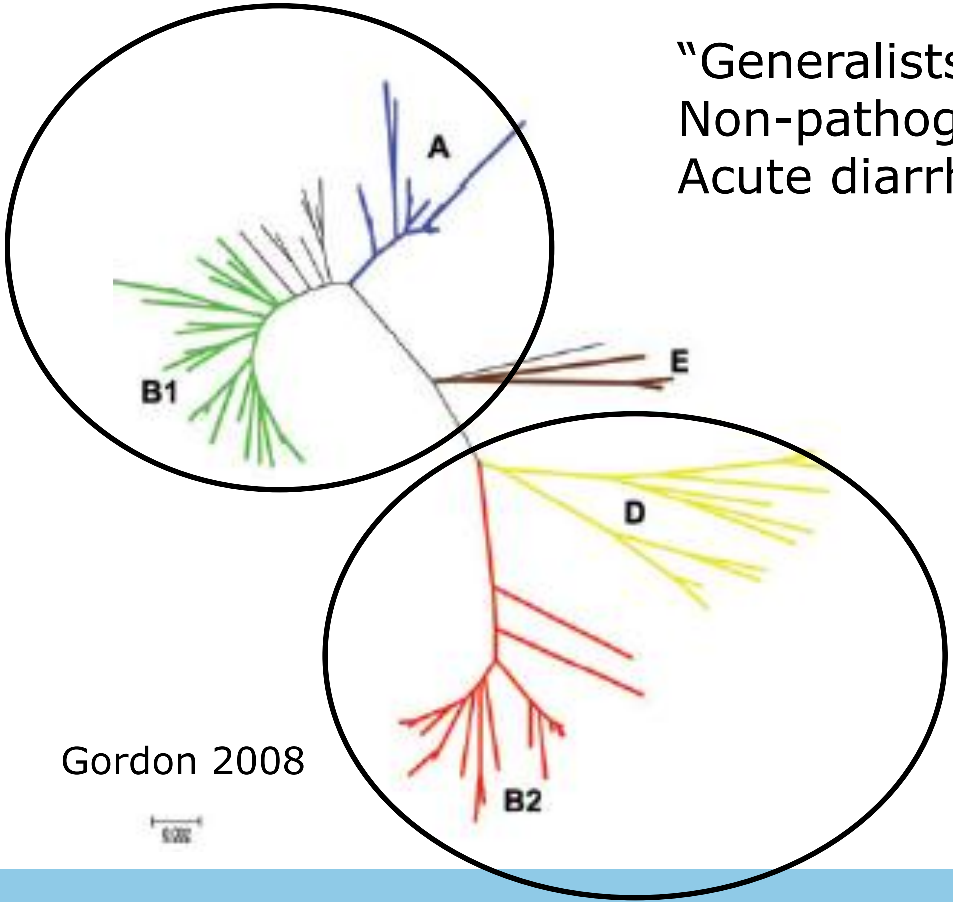
Stress resistance & metabolism: gaps

- What is the niche for selecting for *rpoS* mutants?
  > Human?
- What is the selective advantage of *rpoS* mutants?
  - Increased nutrient scavaging?
    - › Enhanced expression central metabolism (TCA/Krebs) (Ferenci 2003)
    - › Increased respiration on non-preferred nutrient sources
      - L-threonine, L-serine (Dong 2009, Franz 2011, Parker 2012)
      - Succinate, fumarate, malate (intermediates central metabolism) (Dong 2009)
    - › Increased expression of LEE genes (*tir*, *espA*) (Dong 2009)



E. coli phylogroups

Differ in ecological niche, life-history traits, disease potential



"Generalists"
Non-pathogenic commensals
Acute diarrheal disease (STEC, ETEC, EIEC)

"Specialists"
Chronic/mild diarrheal disease (D)
(EPEC, EAEC, DAEC)
More extra-intestinal virulence genes

Gordon 2008

Meric 2012
Walk 2007
White 2011

E. coli phylogroups in the environment

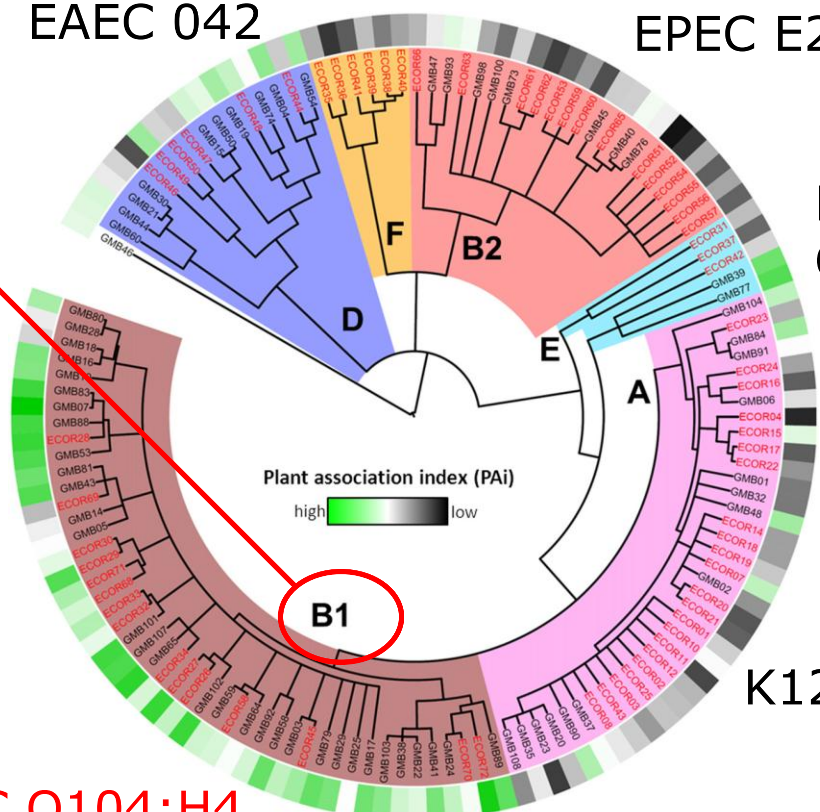
- Environmental persistence
- Plant associated
    - Higher biofilm production
    - Higher metabolism on plant sugars
    - **Higher RpoS levels**

EAEC 042

UPEC CFT073
EPEC E2348/69

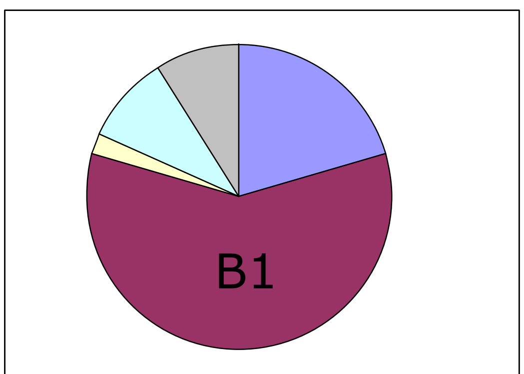
EHEC O157

K12 MG1655



EAHEC O104:H4

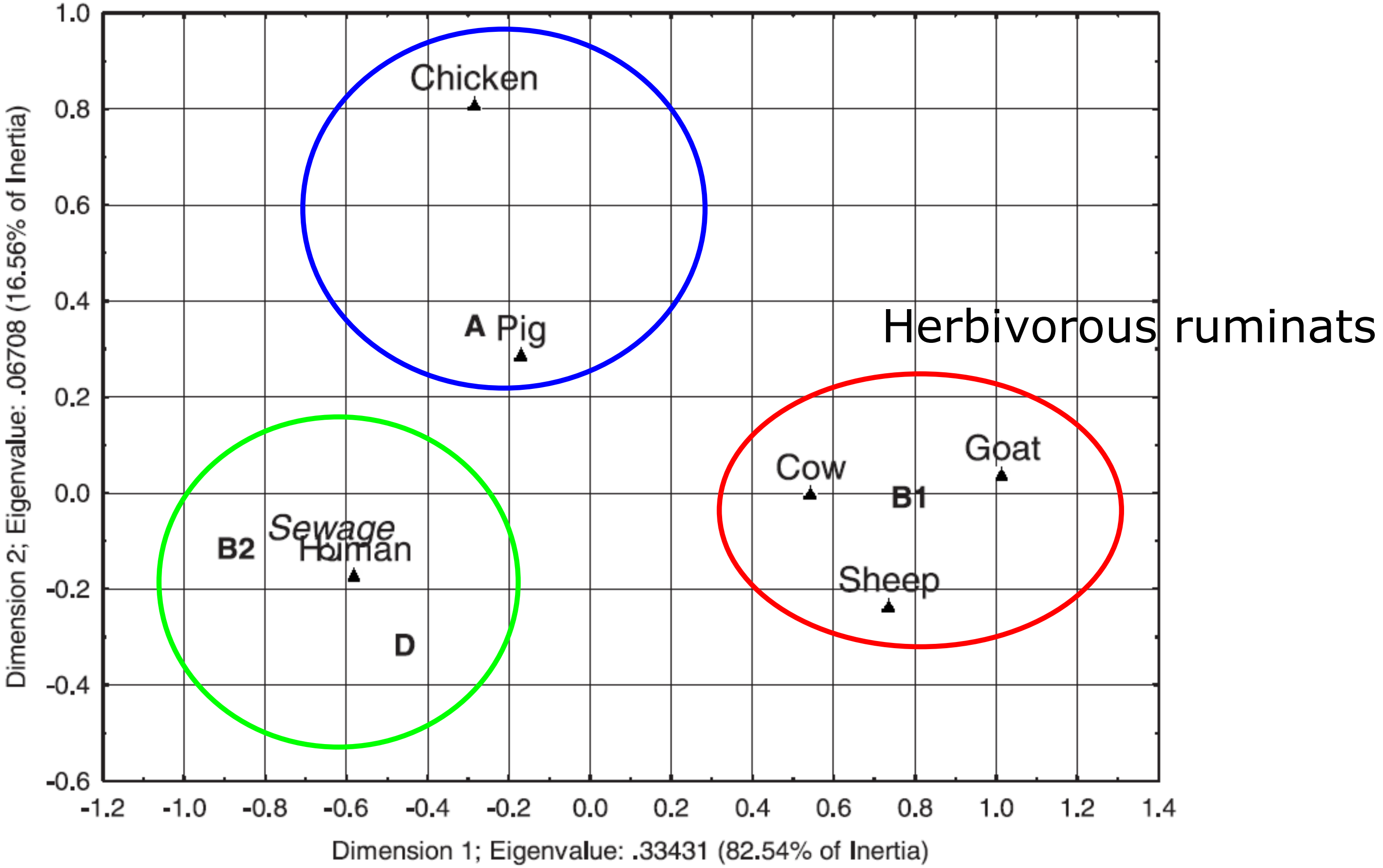
Non-O157 STEC (n=225)
60% B1, 20% A



Franz unpubl.

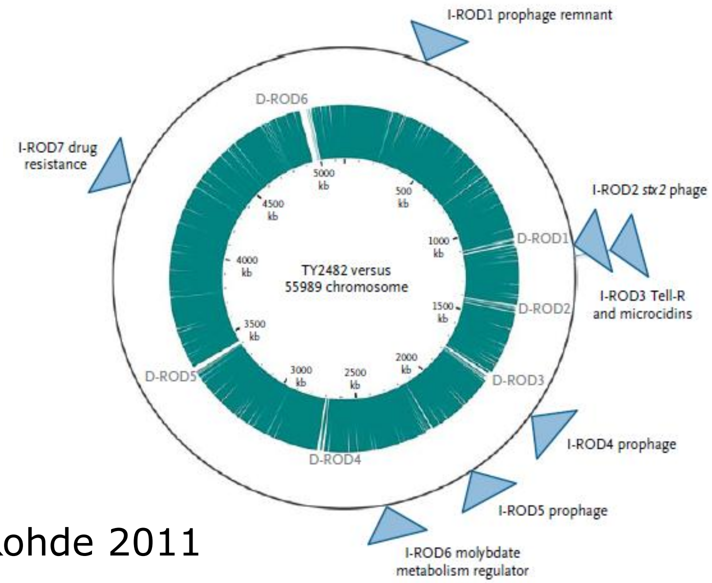
Carlos 2011

E. coli phylogroups origin



From genomics (back) to phenotype

- Increased possibilities genome sequencing: wealth of genomic data
  - Strain identification, genetic markers, evolutionary reconstruction
- Difficult if not impossible to prediction of phenotype (complex regulatory networks)



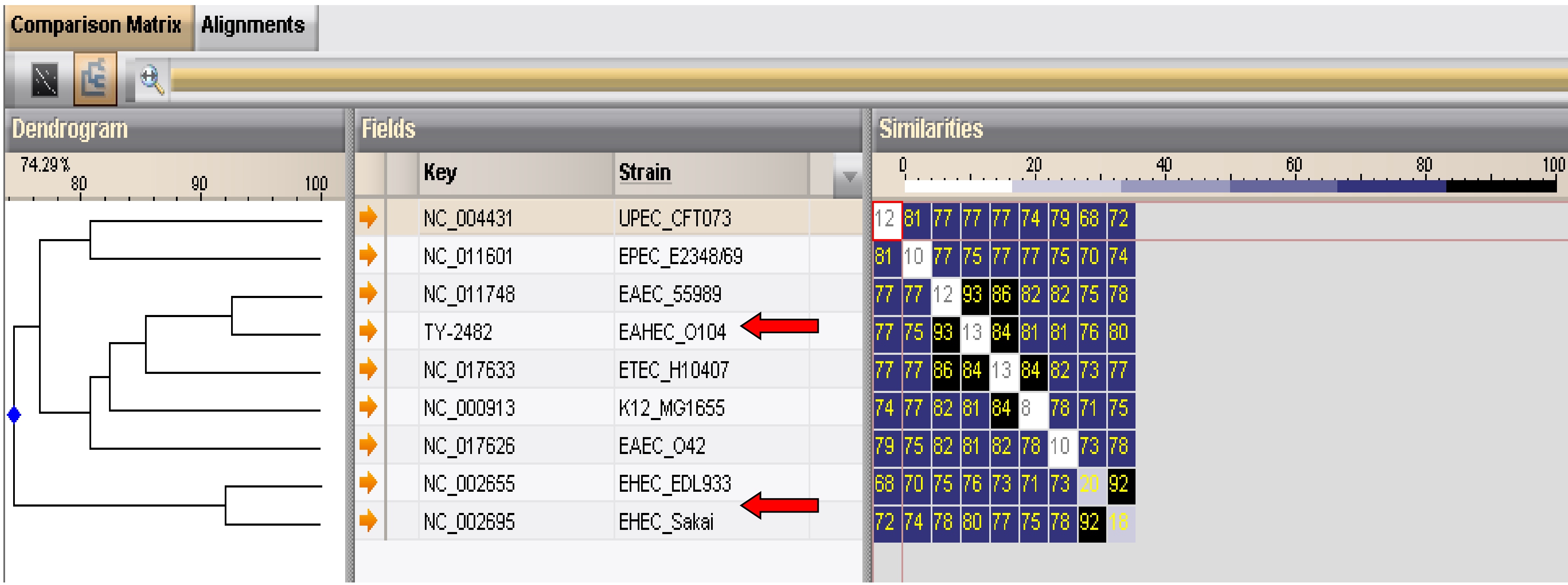
Rohde 2011

In vivo / in vitro:
Virulence
Metabolism
Fate in environment, food

?

Franz in prep.

Comparative genomics and metabolic profiling of HUSEC O104:H4



Comparative metabolic profiling of HUSEC O104:H4

Pathotype	Serotype	Strain	Phylogentic group	Source	Sequence reference
Commensal	OR:H48	K12_MG1655	A	Wild-type laboratory strain of *E. coli* K-12 (Blattner 1997)	Blattner 1997
UPEC	O6:H1	CFT073	B2	USA, Maryland University Hospital (Manges 2001)	Welch 2002
ETEC	O78:H11	H10407	A	Bangladesh prior to 1973, adult with cholera-like symptoms (Evans 1973, Evans 1987, Coster 2006)	Crossman 2010
EPEC	O127:H6	E2348/69	B2	UK 1963, infant with diarrhea	Iguchi 2009
EHEC	O157:H7	EDL933	E	USA 1982, clinical isolate implicated in hamburger outbreak (Riley 1983, Wells 1983).	Perna 2001
EHEC	O157:H7	Sakai	E	Japan 1996, clinical isolate implicated in radish sprout outbreak (Michino 1999)	Hayashi 2001
EAEC	O44:H18	042	D	Peru 1983, child with diarrhea (Nataro 1995)	Chaudhuri 2010
EAHEC	O104:H4	586	B1	Netherlands 2011, adult patient linked to German outbreak associated with fenugreek (this study)	Rohde 2011

Franz in prep.

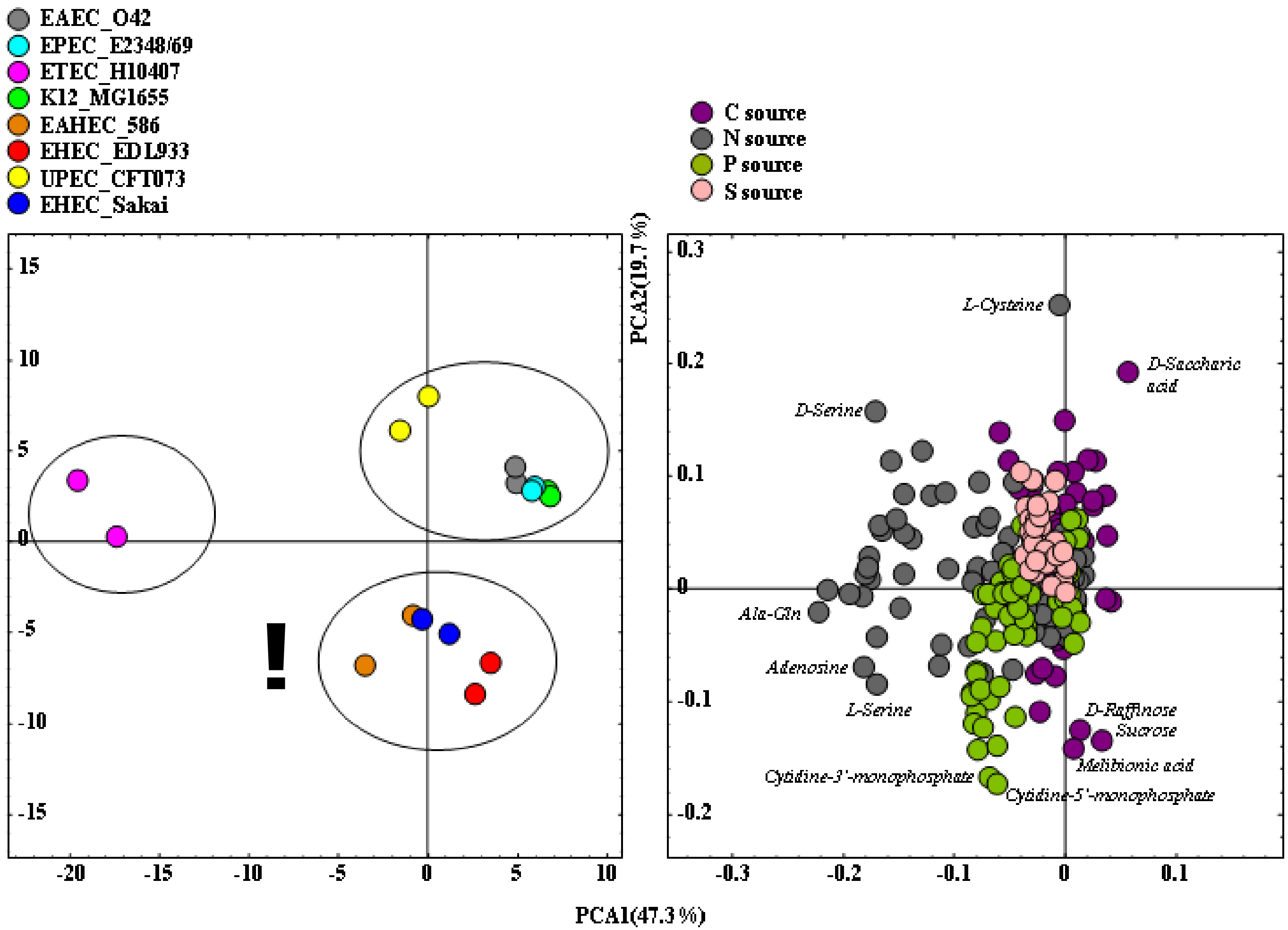
Comparative metabolic profiling of HUSEC O104:H4

Mean metabolic activities

	K12 MG1655	EAEC O42	ETEC H10407	EPEC E2348/69	EAHEC O104:H4	EHEC EDL933	EHEC Sakai	UPEC CFT073
ALL	1.36±0.76 [a]	1.47±0.76 [a, b]	**2.03±1.50 [d]**	1.47±0.73 [a]	1.73±0.91 [c]	1.69±0.95 [b, c]	1.43±0.97 [a]	**1.88±0.91 [c, d]**
C	1.40±0.88 [a]	1.45±0.87 [a]	1.51±0.87 [a, b]	1.48±0.85 [a, b]	1.57±0.76 [a, b]	**1.76±0.93 [b, c]**	1.31±0.80 [a]	**1.88±0.76 [c]**
N	1.18±0.78 [a]	1.42±0.82 [a, b, c]	**2.77±2.34 [d]**	1.38±0.78 [a, b]	1.94±1.10 [c]	1.39±0.83 [a, b]	1.41±1.29 [a, b, c]	1.75±1.10 [b, c]
P	1.56±0.39 [a]	1.60±0.33 [a]	**2.62±1.05 [c]**	1.53±0.26 [a]	**2.25±0.92 [b, c]**	**2.38±1.00 [b, c]**	2.04±0.89 [b]	**2.23±0.92 [b, c]**
S	1.25±0.28 [b, c]	1.48±0.34 [c, d]	**1.82±0.50 [e]**	1.48±0.30 [c, d]	1.12±0.26 [a, b]	0.93±0.15 [a]	1.14±0.035 [a, b]	**1.59±0.26 [d, e]**

Franz in prep.

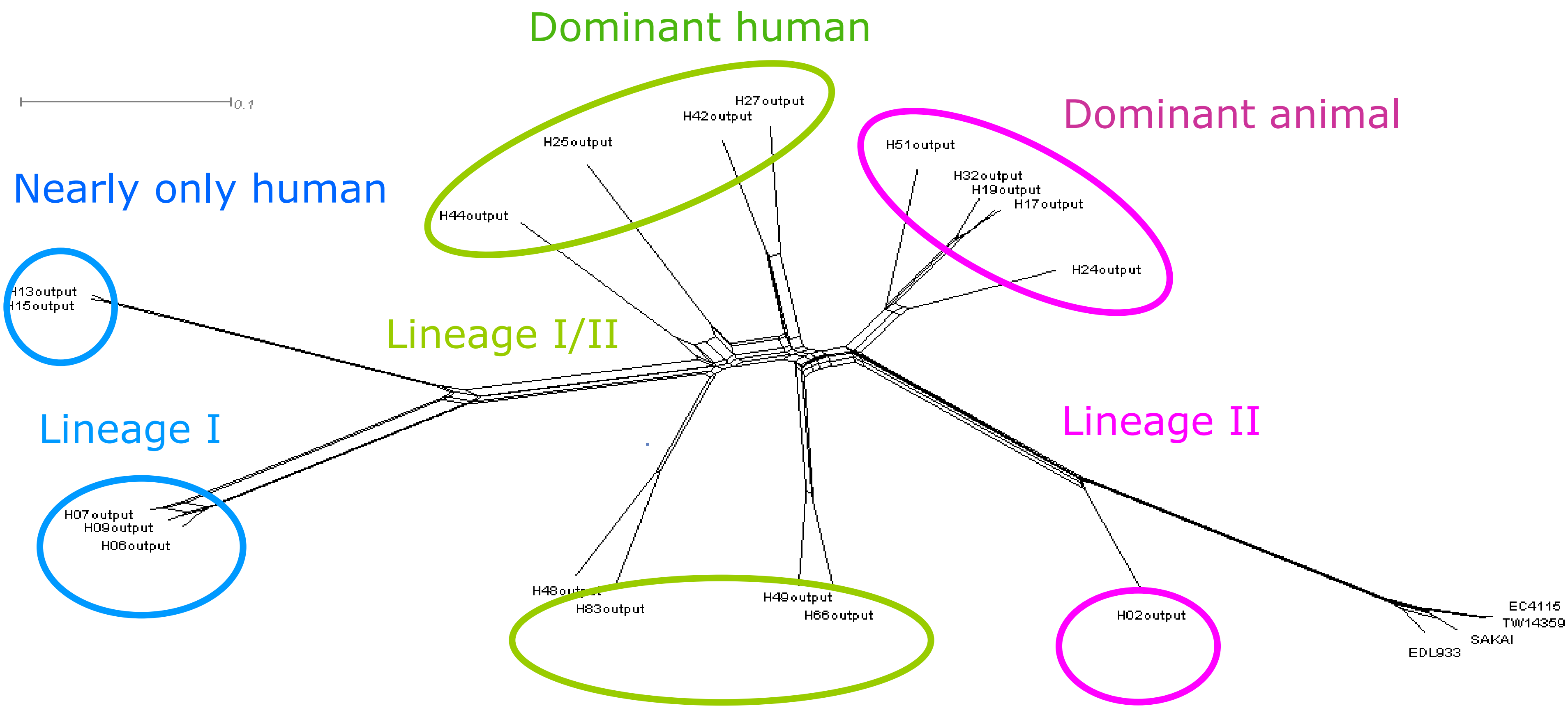
Comparative metabolic profiling of HUSEC O104:H4



Franz and van Hoek, in prep.

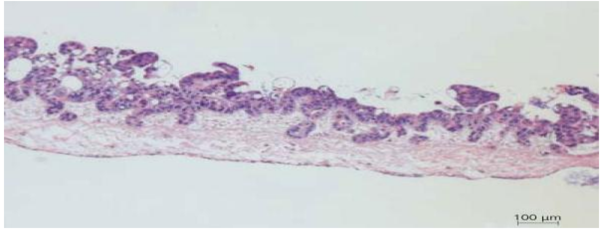
Sequencing Dutch O157 strains

Non-random distribution lineages over sources → WHY?

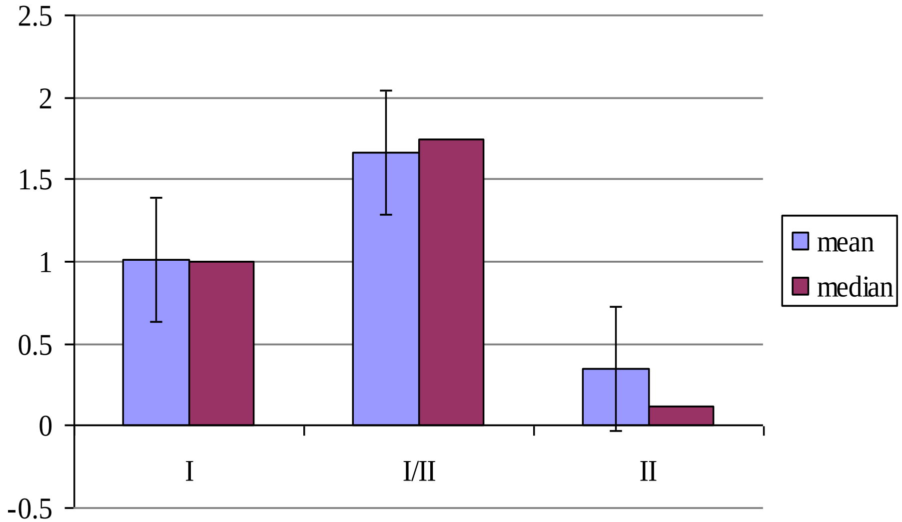


Franz in prep.

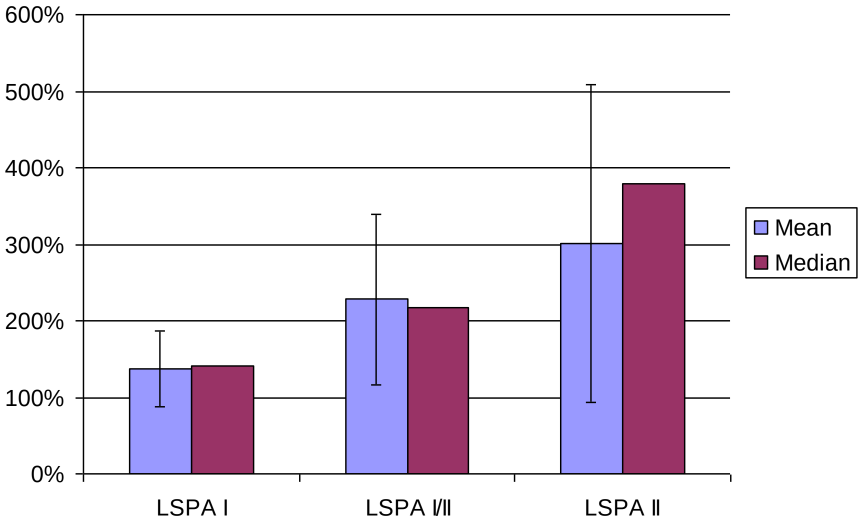
Relation to phenotype



Stx production



Adherence CIEB / CACO

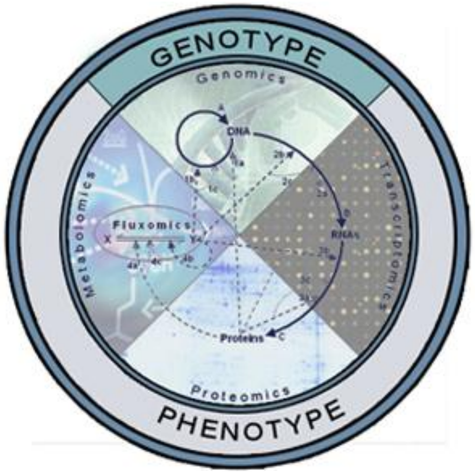


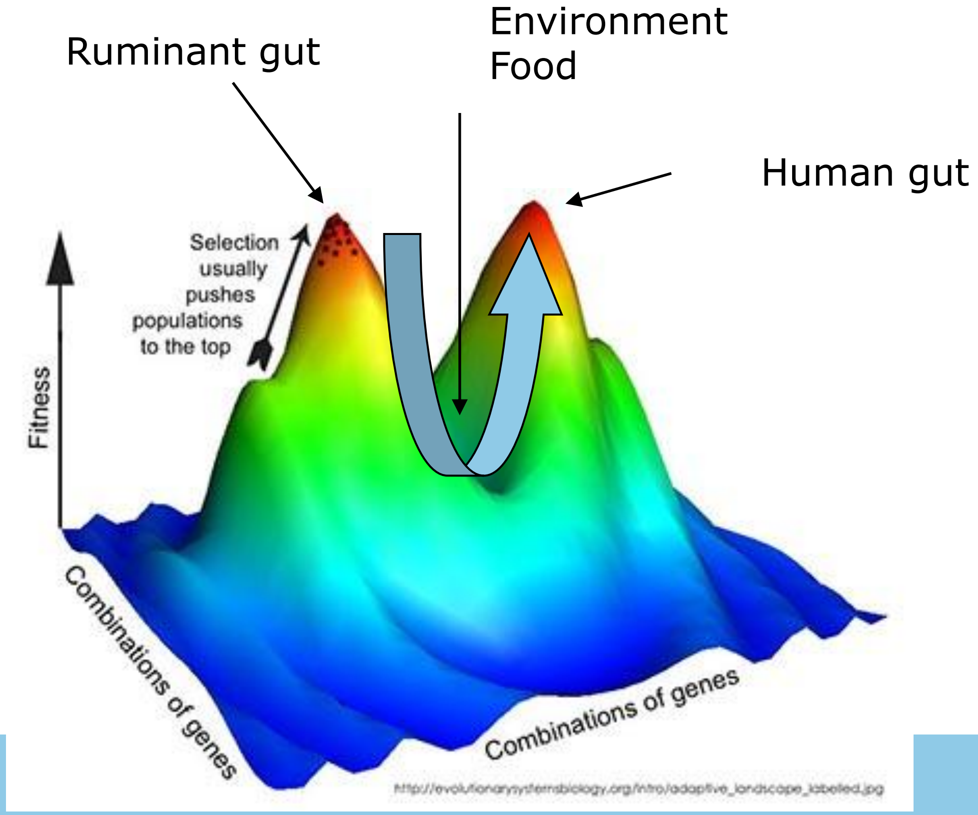
Biolog metabolic profiling
Transmission: survival environment, food (plant)?

Take home message (1/2)

1. Stress resistance regulation and metabolic capacity are dynamic processes within STEC populations and seem vital for persistence in host and non-host environments.

2. Do not forget the phenotype





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