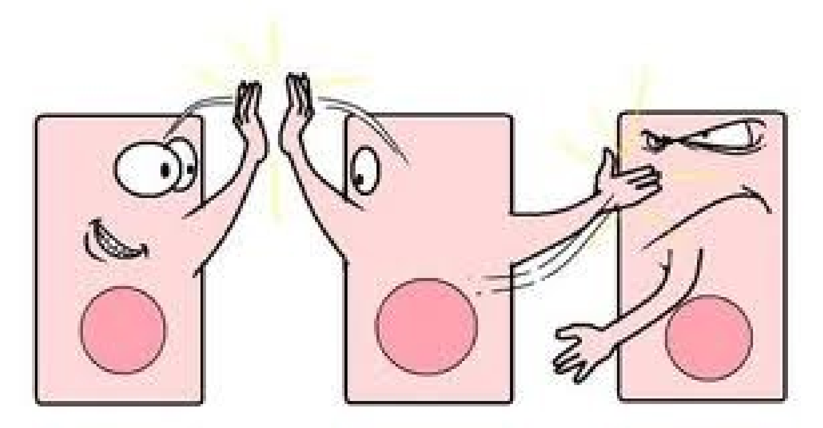
EHEC sings: pour some sugar on me

Vanessa Sperandio
Depts. Microbiology and Biochemistry
UT Southwestern Medical Center
Dallas TX

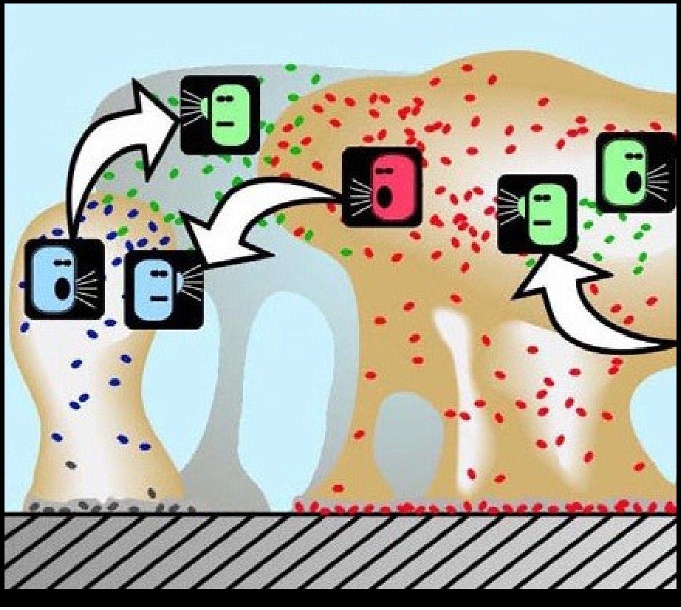
Cell-to-cell chemical communication

Multi-cellular organisms



To coordinate many aspects of physiology, behavior and development through chemical communication

This cell-to-cell chemical communication is achieved using several chemical languages that are the hormones

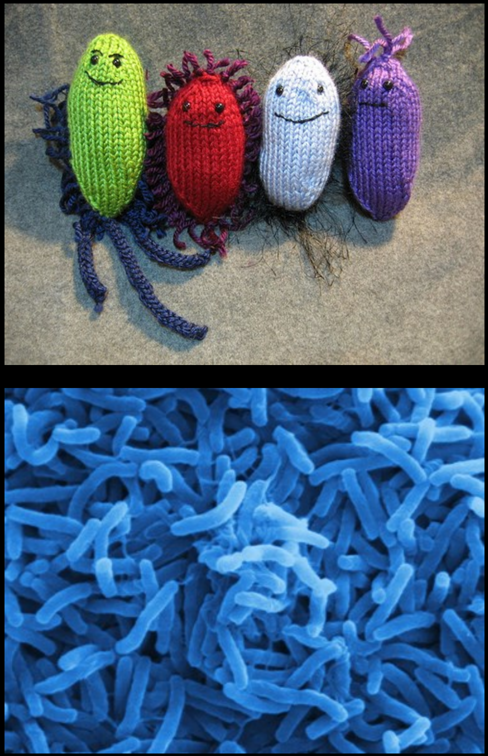


Bacterial cells also communicate with each other through chemicals to coordinate population behavior

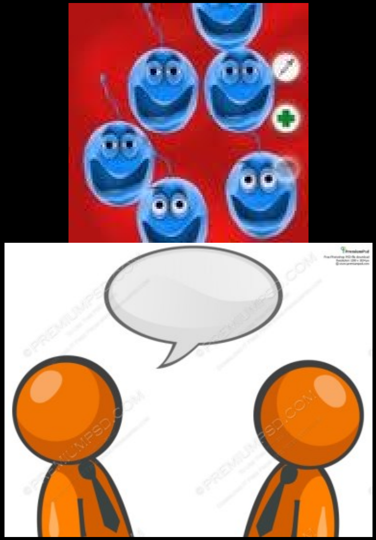
Chemical signaling in bacteria was discovered in 1970 (Nealson And Hastings), and named Quorum sensing in 1994 (Fuqua, Greenberg and Winans)
The chemicals are called autoinducers (AIs)

Inter-kingdom chemical signaling

Bacterial perform chemical communication through autoinducers



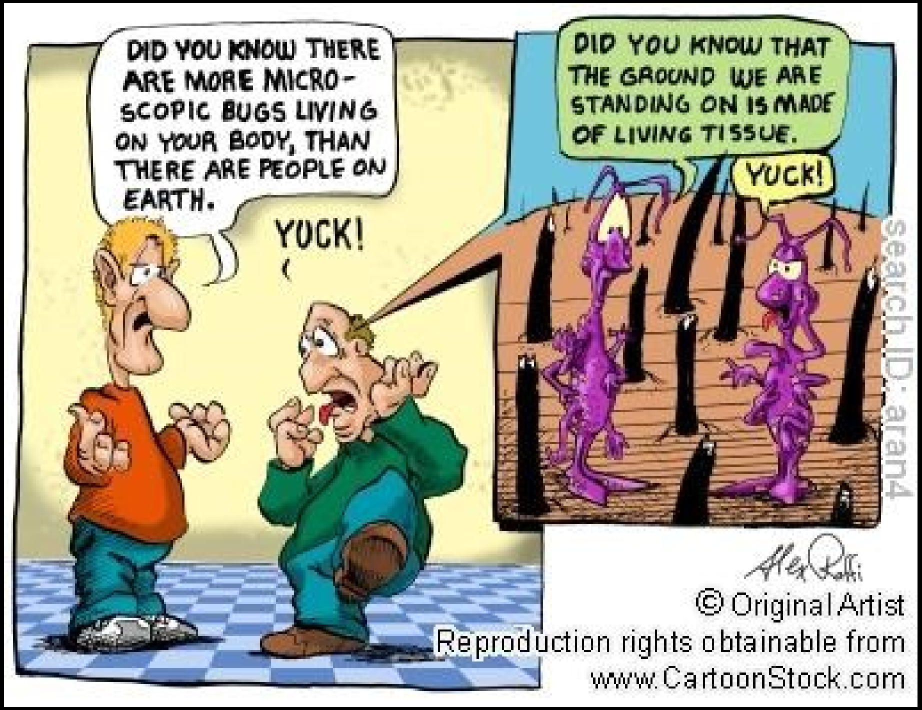
Animal cells perform chemical communication through hormones



Inter-kingdom chemical signaling intersects bacterial autoinducers and mammalian hormones to bacteria and mammals can communicate

You are more microbial than human

- Human cells 10^{13} ~35,000 genes
- Gastrointestinal tract 10^{14} bacterial cells ~1,000,000 genes
- Indigenous microbiota plays an important role in nutrient assimilation and production of vitamins
- An important role in the development of the mammalian digestive, physiology and immune systems
- Microbes modulate expression of mammalian genes

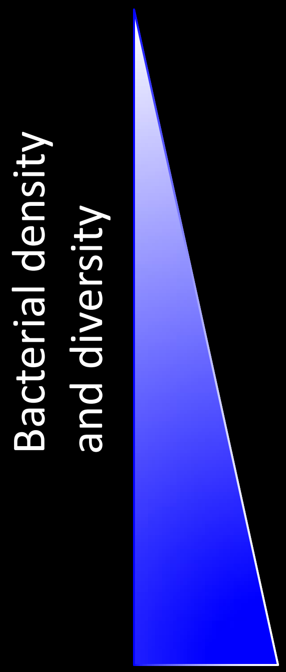


To have a quorum you need chemicals

But you can't just leave on chemistry
You need Food!

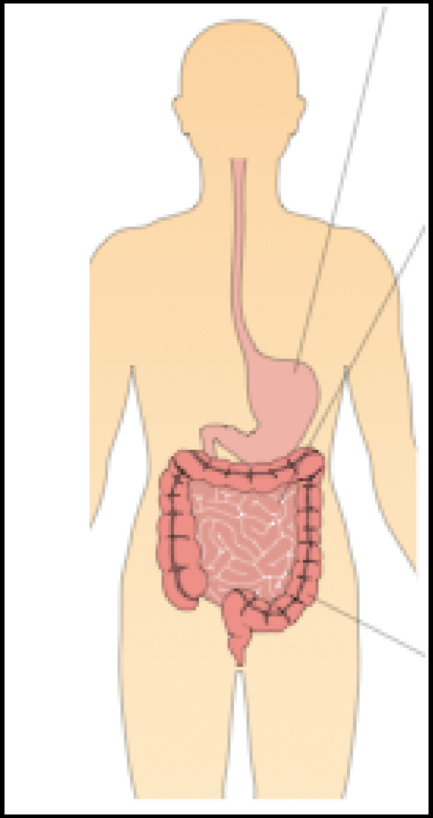
The ability to sense chemicals and food can bring us together

Human gut is a complex and highly populated environment: 10-100 trillions of commensal bacteria



Small intestine 10^7CFU/mL

Large intestine 10^{11}CFU/mL



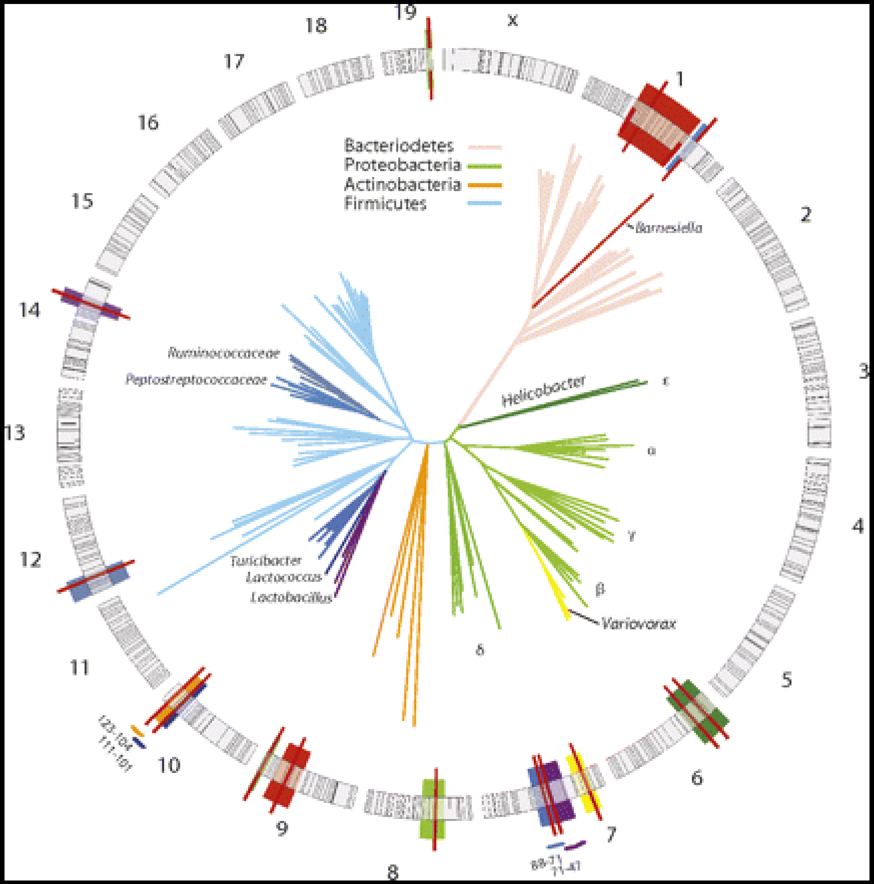
- Intestinal microbiota is abundant and diverse (estimated 500-1000 species)

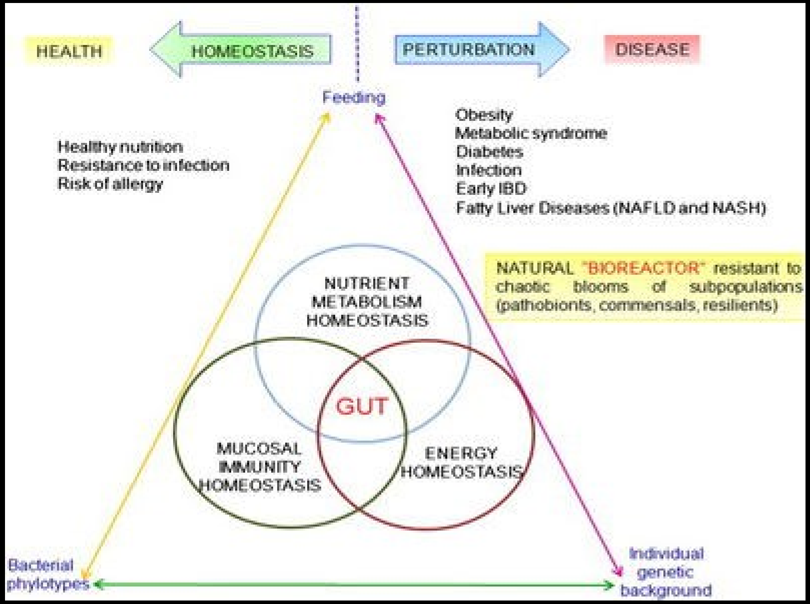
- *Firmicutes* and *Bacteroidetes* are predominant

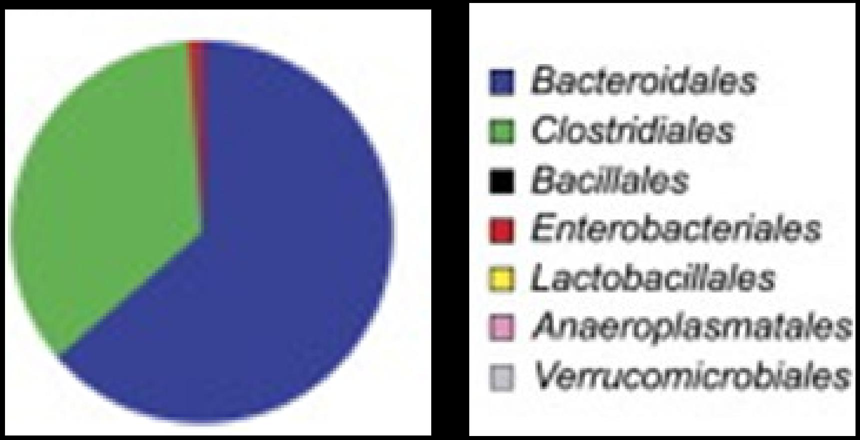
- Chemical signaling in the GI tract helps to coordinate population behavior

- Carbon and nitrogen utilization capabilities play a major role for homeostasis of commensal bacteria and infection by pathogenic species

Intestinal microbiota composition



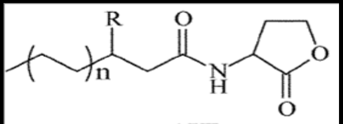




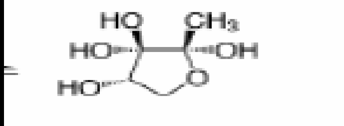
Inter-kingdom chemical signaling employ multiple info-chemicals (signals and nutrients)

Bacterial derived signals produced by the microbiota

AI-3 structure unknown derived from aromatic Metabolism.

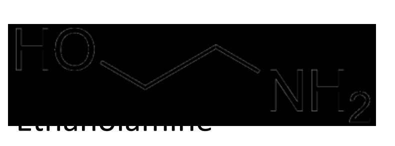


AHL



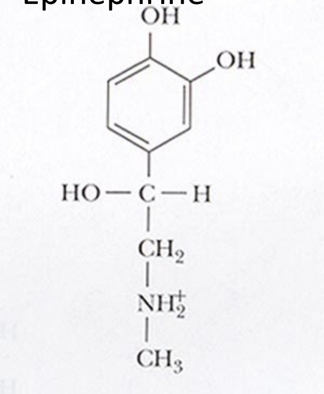
(2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF)

Nutrients (C and N sources)

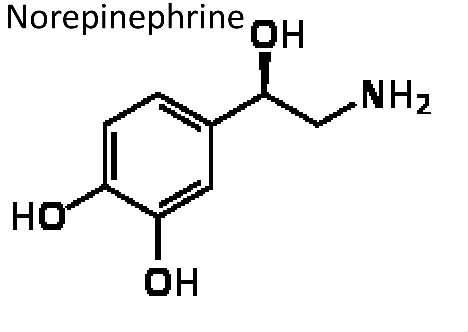


Host signals

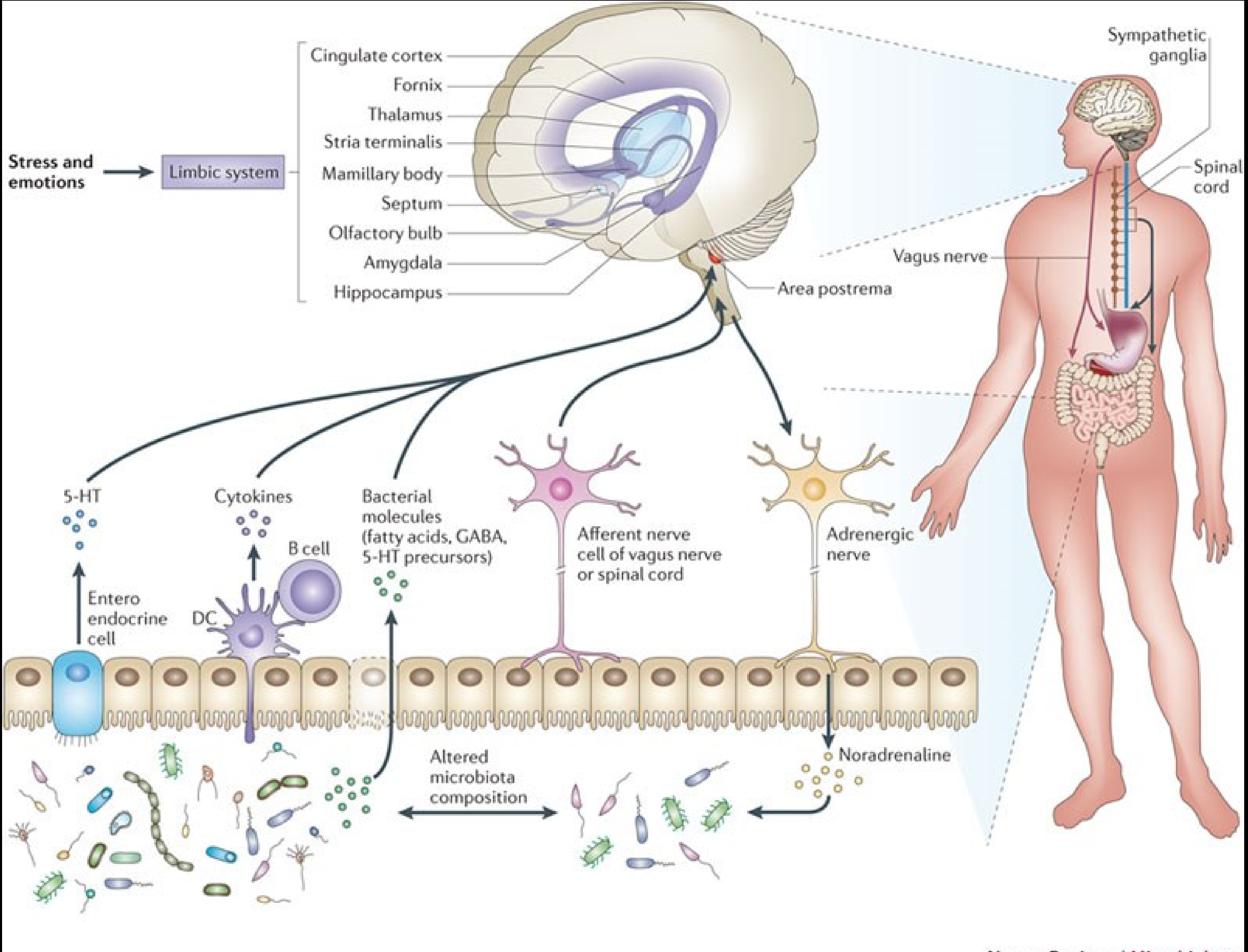
Epinephrine



Norepinephrine

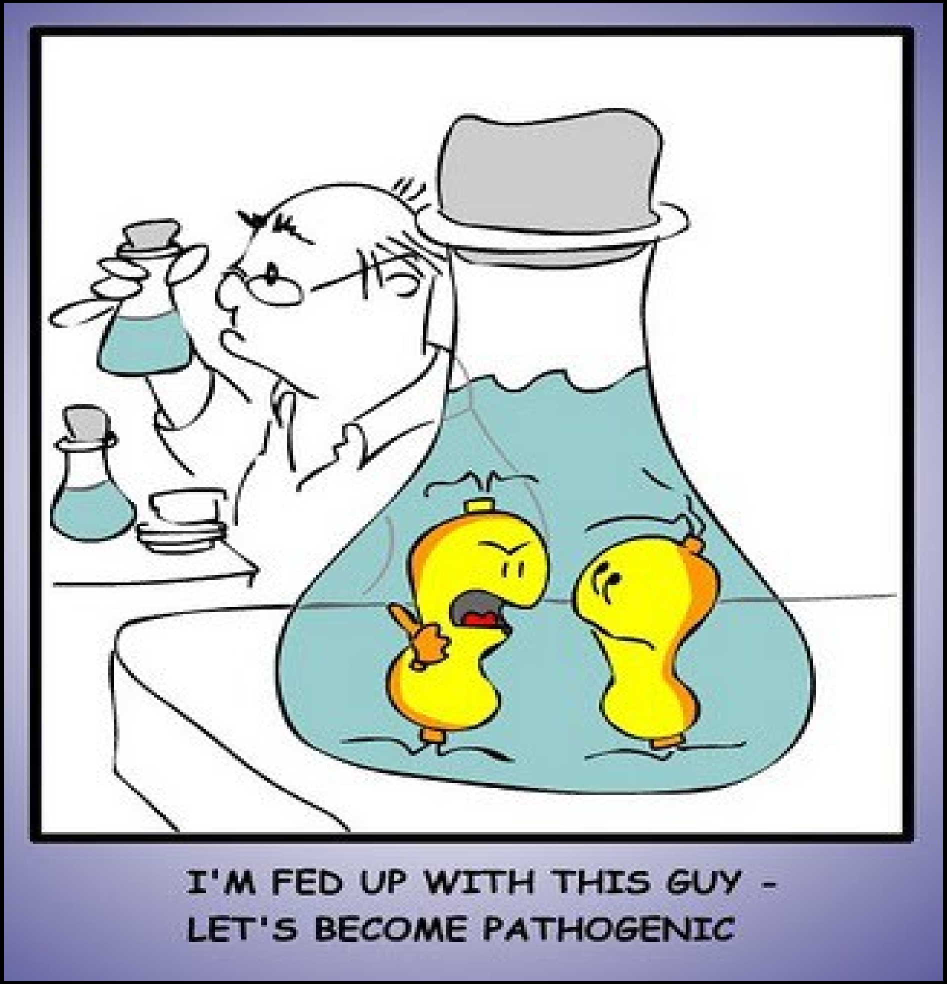


- Epinephrine and norepinephrine are the stress “fight or flight” hormones
- NE produced by adrenergic neurons ENS
- Epi, produce CNS adrenal medulla: systemic effect through bloodstream
- NE/Epi control intestinal motility
- Cl^- and K^+ secretion
- NE/Epi control the immune system

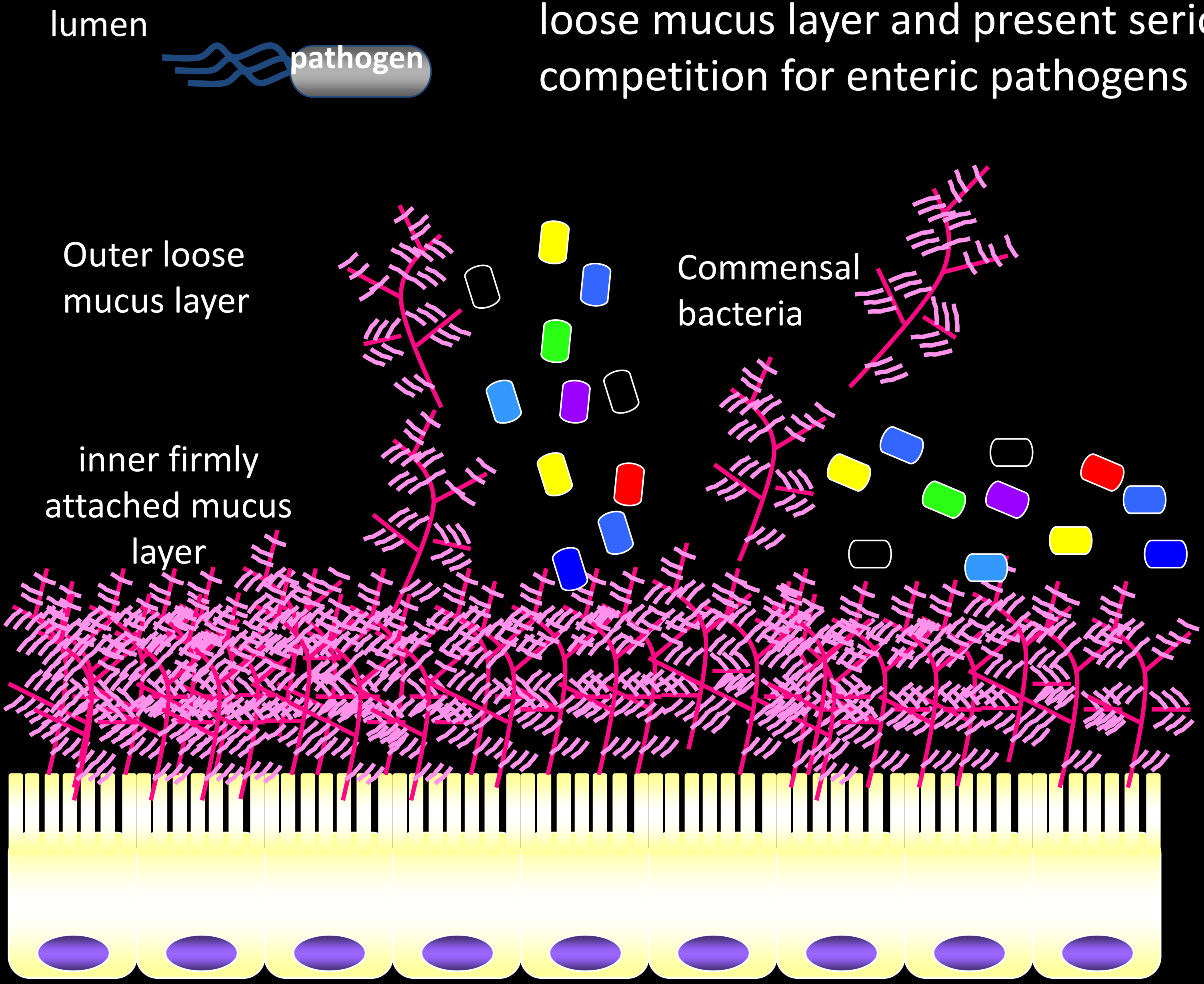


Nature Reviews | Microbiology

Although these bacterial/host infochemicals (signals and nutrient cues) associations evolved towards co-operation, they can be exploited by bacterial pathogens to cause disease.



Commensal bacteria live in the outer loose mucus layer and present serious competition for enteric pathogens



lumen

pathogen

Outer loose mucus layer

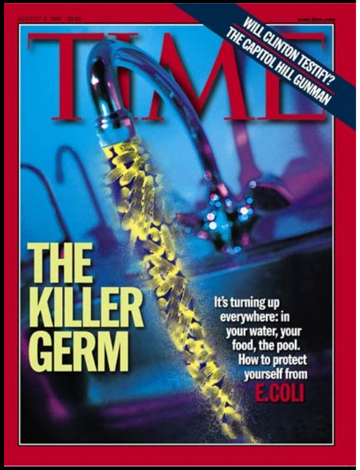
Commensal bacteria

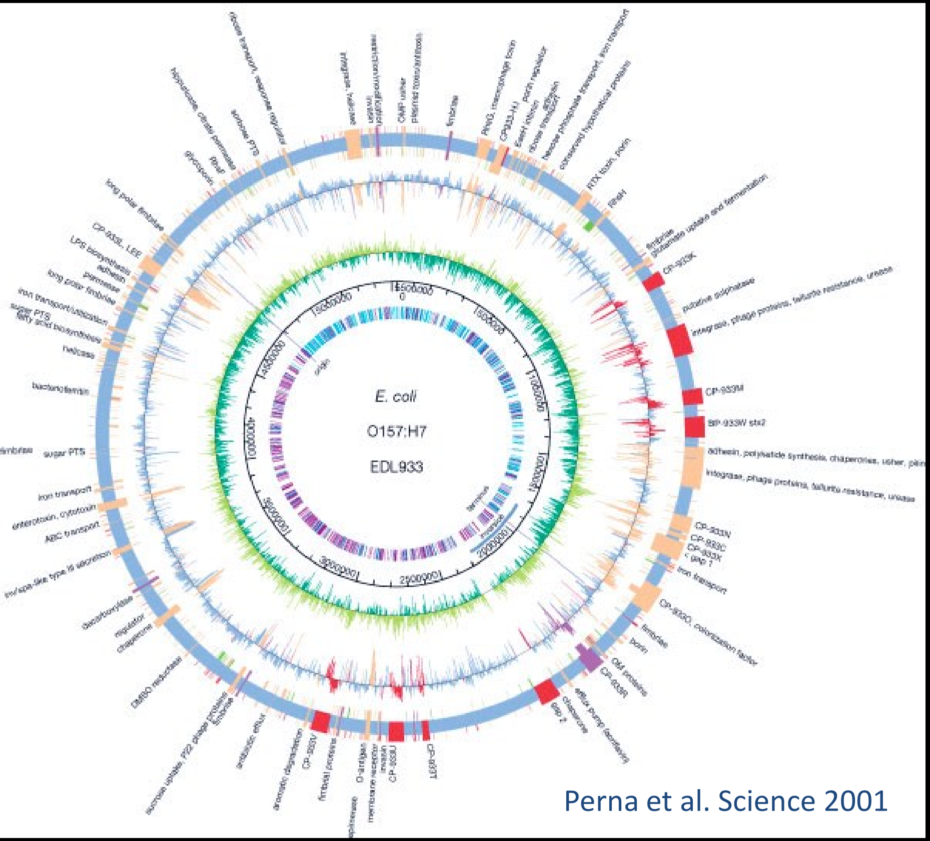
inner firmly attached mucus layer

Intestinal epithelium

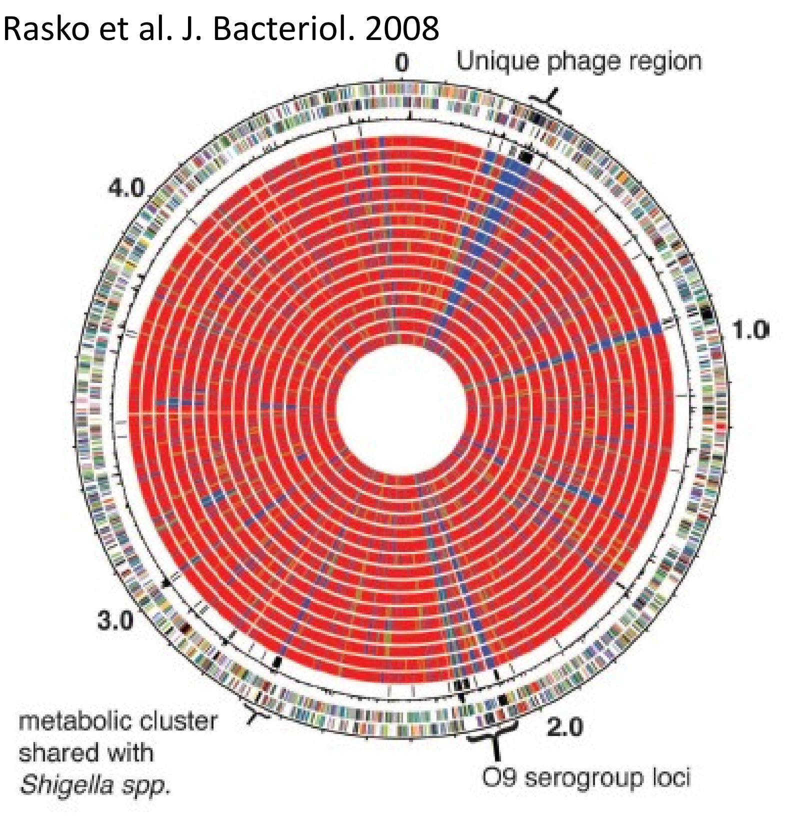
Enterohemorrhagic *E. coli* (EHEC) O157:H7

- Responsible for outbreaks of bloody diarrhea in many countries
- Higher probability of developing <u>H</u>emolytic <u>U</u>remic <u>S</u>yndrome (HUS) in the young and elderly.
- Infection by contaminated food and water
- Low Infection dose (50-100 cfu)
- Colonizes the large intestine





Perna et al. Science 2001



Rasko et al. J. Bacteriol. 2008

Between all *E. coli* genomes
Only 2,200 genes are shared
(E. coli genomes vary between
4,000 to 5,000 genes)

Pangenome of *E. coli* has a
gene repertoire of 13,000

Rasko et al. J. bacteriol 2008

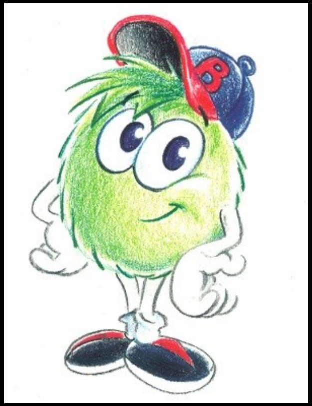
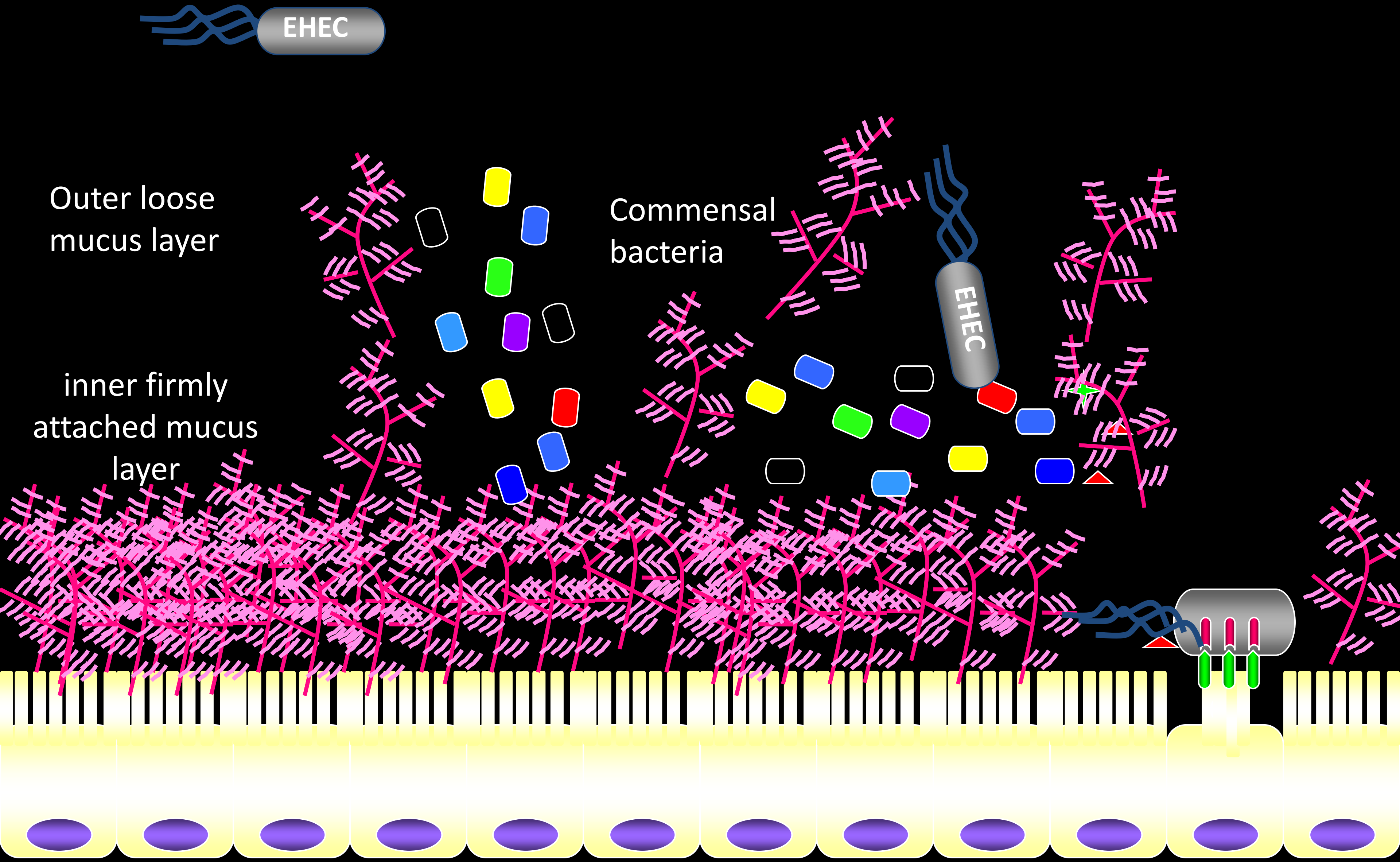


illustration: Don Smith

lumen



Outer loose mucus layer

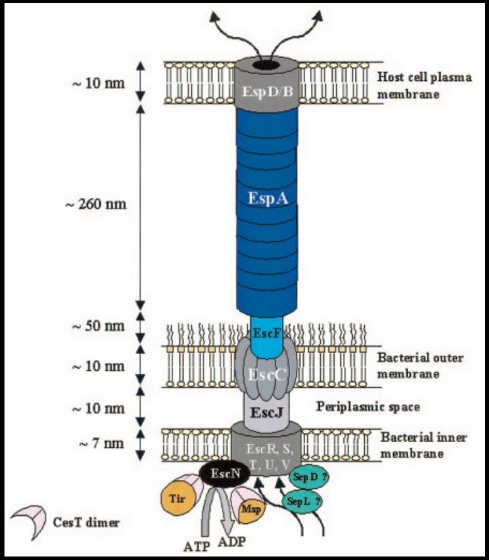
Commensal bacteria

inner firmly attached mucus layer

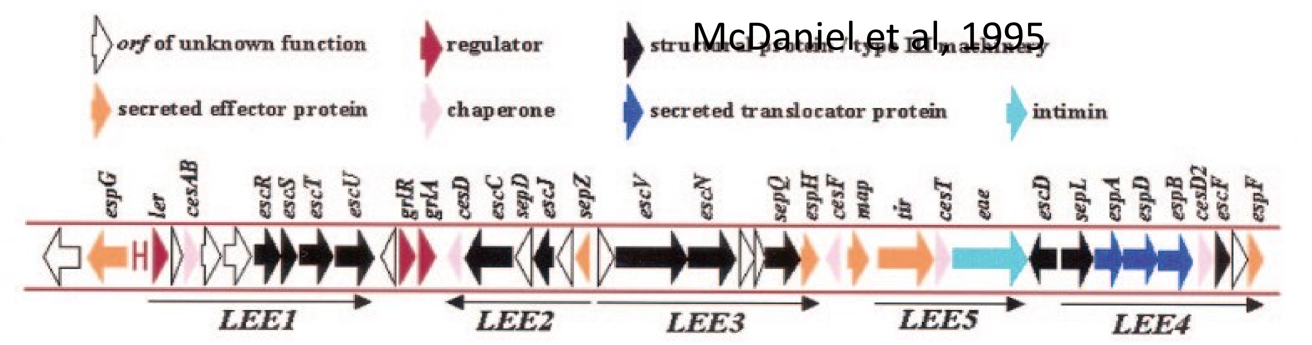
Intestinal epithelium

<u>L</u>ocus of <u>E</u>nterocyte <u>E</u>ffacement (*LEE*)

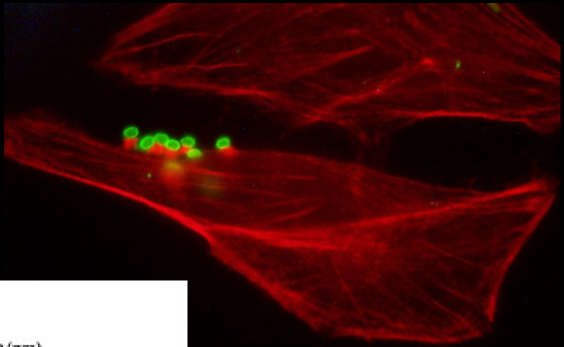
T3SS of EHEC



Garmendia et al. , 2005. *Infect Immun*



McDaniel et al, 1995



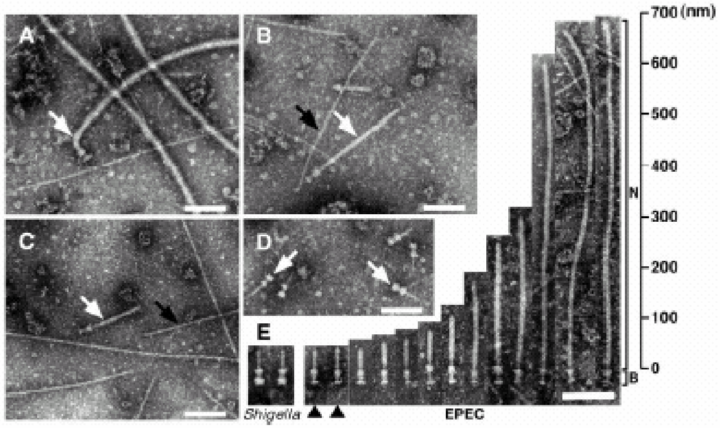
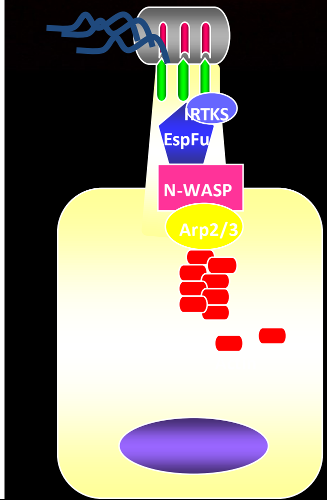
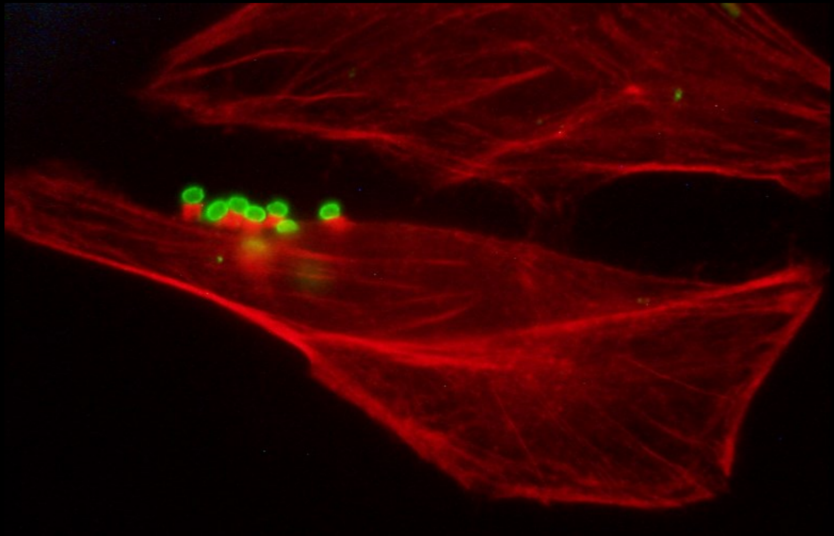
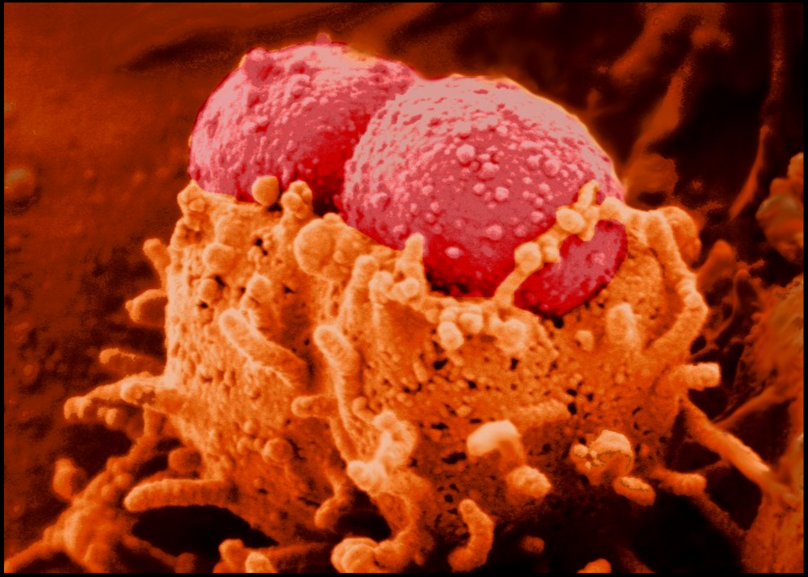


Fig. 1. Electron micrographs of negatively stained NC fractions from EPEC and *Shigella*. The NCs were partially purified from EPEC E2348/69 strain grown in LB broth (*A*), in DMEM (*B*), and from EPEC B171–8 grown in DMEM (*C*). (*D*) Electron micrograph of purified *Shigella* NC. (*E*) Alignment of EPEC NCs and comparisons to *Shigella*. N and B indicate the needle and basal body of EPEC NCs, respectively. Black arrowheads indicate putative immature NCs. White arrows indicate flagellar complexes (*A*) and NCs (*B*, *C*, and *D*). Black arrows indicate pilus-like structures. (Bars = 100 nm.)



Attaching and effacing lesions or pedestals





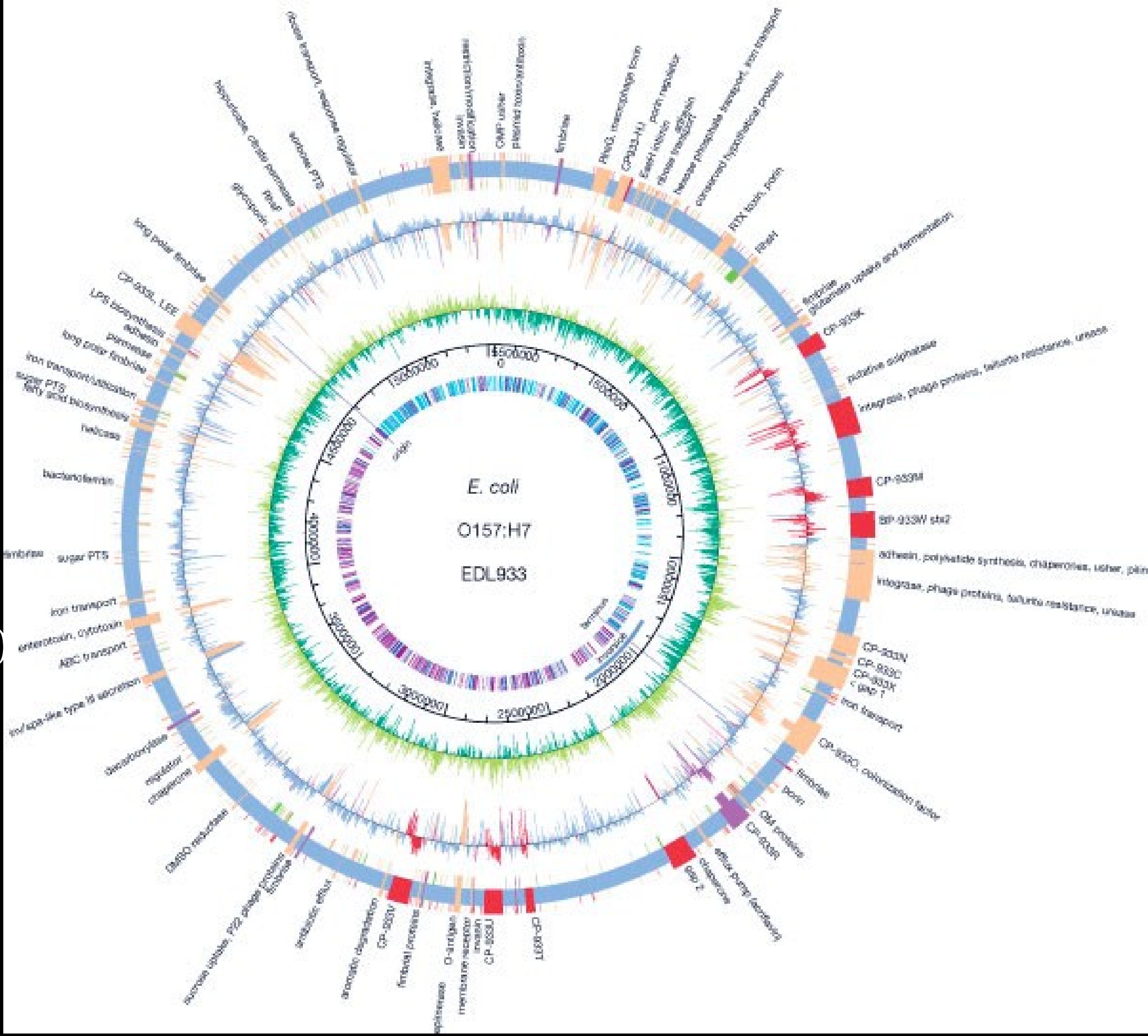
-K-12 genome 4Mb

-EHEC genome 5Mb

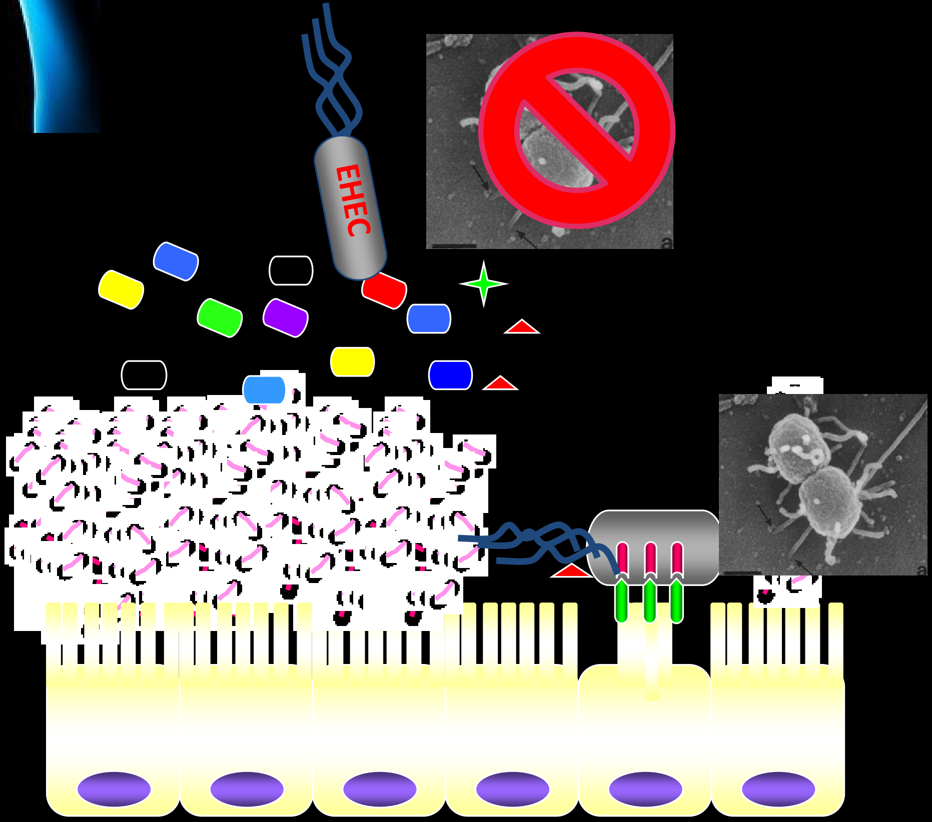
-EHEC has an extra ~1,000 genes

-EHEC has 1.34 Mb of DNA not present in K-12 (pathogenicity islands)

-K-12 has 0.5 Mb Not present in EHEC (black holes)

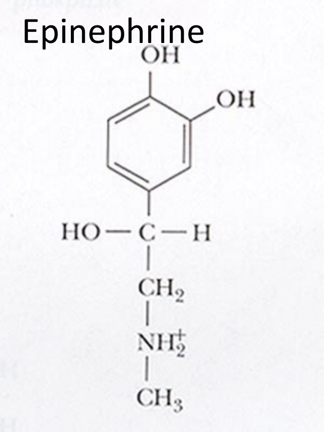


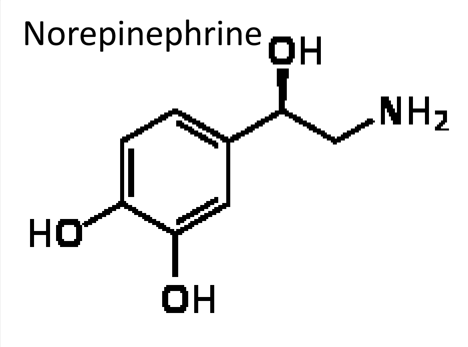
Perna et al. Science 2001


EHEC

To coordinate expression of its virulence repertoire EHEC senses multiple info-chemicals (signals)

Host signals





Bacterial derived signals produced by the microbiota

AI-3 structure unknown derived from aromatic Metabolism. Sperandio et al. PNAS2013

To coordinate expression of its virulence repertoire EHEC senses multiple info-chemicals (nutrients)

Nutrients (C source)

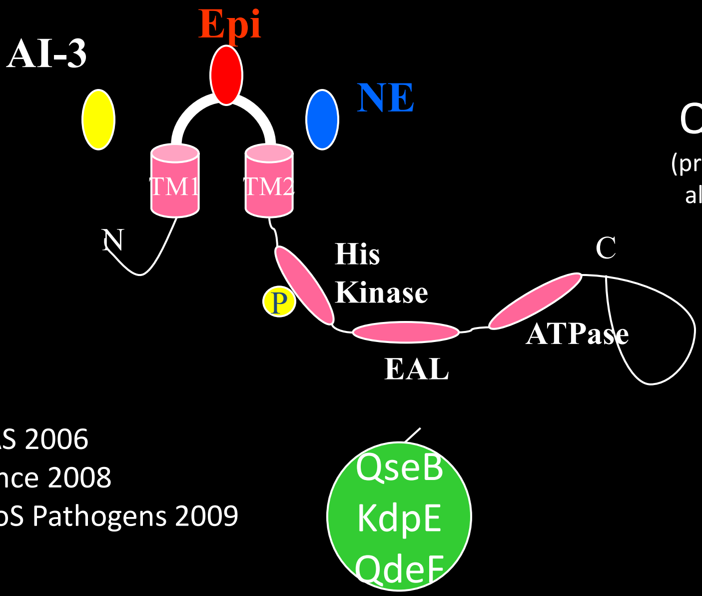
EHEC sings: Pour some sugar on me

QseC

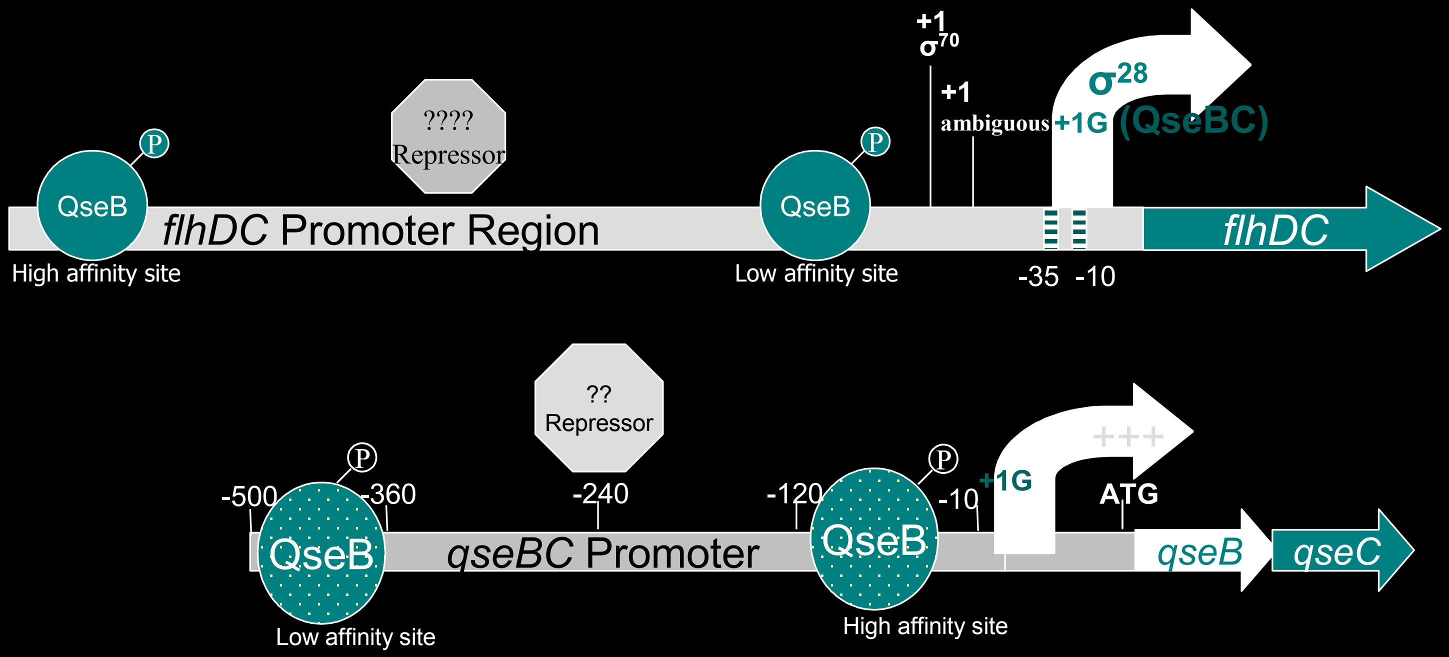
Marcie Clarke

David Hughes

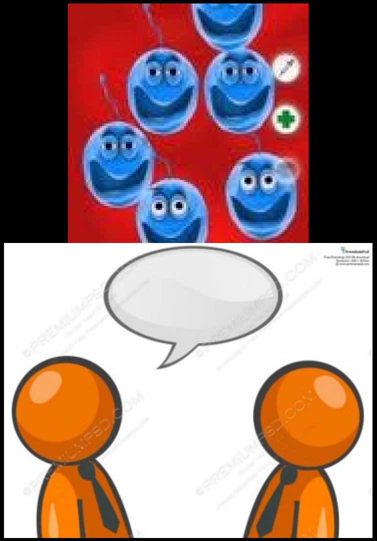
- QseC is an inner membrane bacterial adrenergic receptor that responds to the bacterial signal AI-3 and to the host signals epinephrine and norepinephrine.
- Signaling of QseC leads to autophosphorylation and subsequent phosphotransfer to QseB, QseF and KdpE

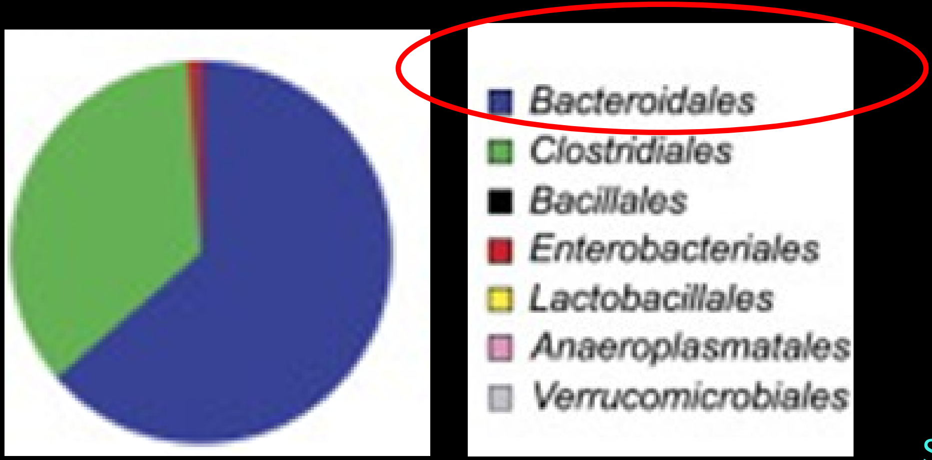


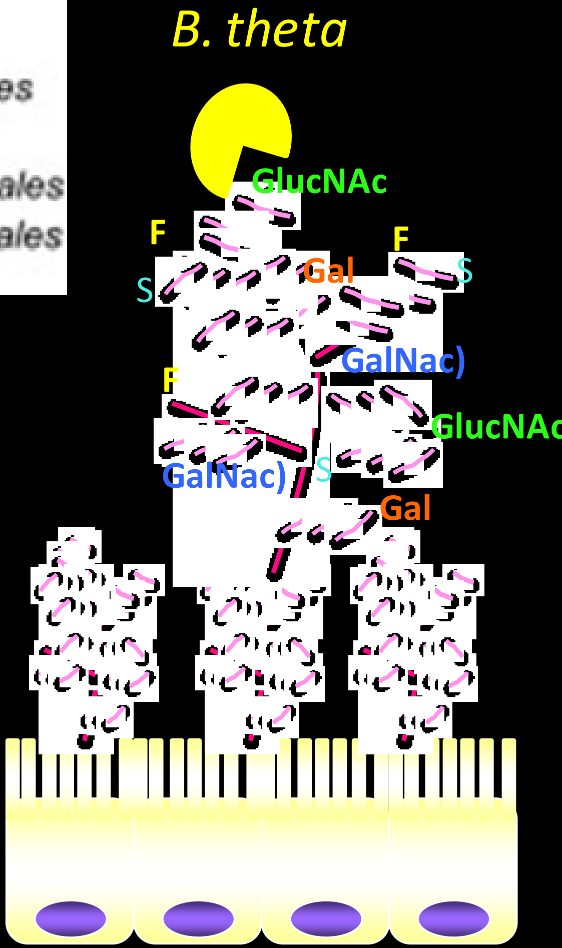
Clarke et al. PNAS 2006
Rasko et al. Science 2008
Hughes et al. PLoS Pathogens 2009


+1
σ70
+1
ambiguous
+1G
σ28
(QseBC)
????
Repressor
P
QseB
flhDC Promoter Region
High affinity site
P
QseB
Low affinity site
-35
-10
flhDC
??
Repressor
P
-500
-360
-240
-120
-10
+1G
+++
ATG
QseB
qseBC Promoter
P
QseB
qseB
qseC
Low affinity site
High affinity site

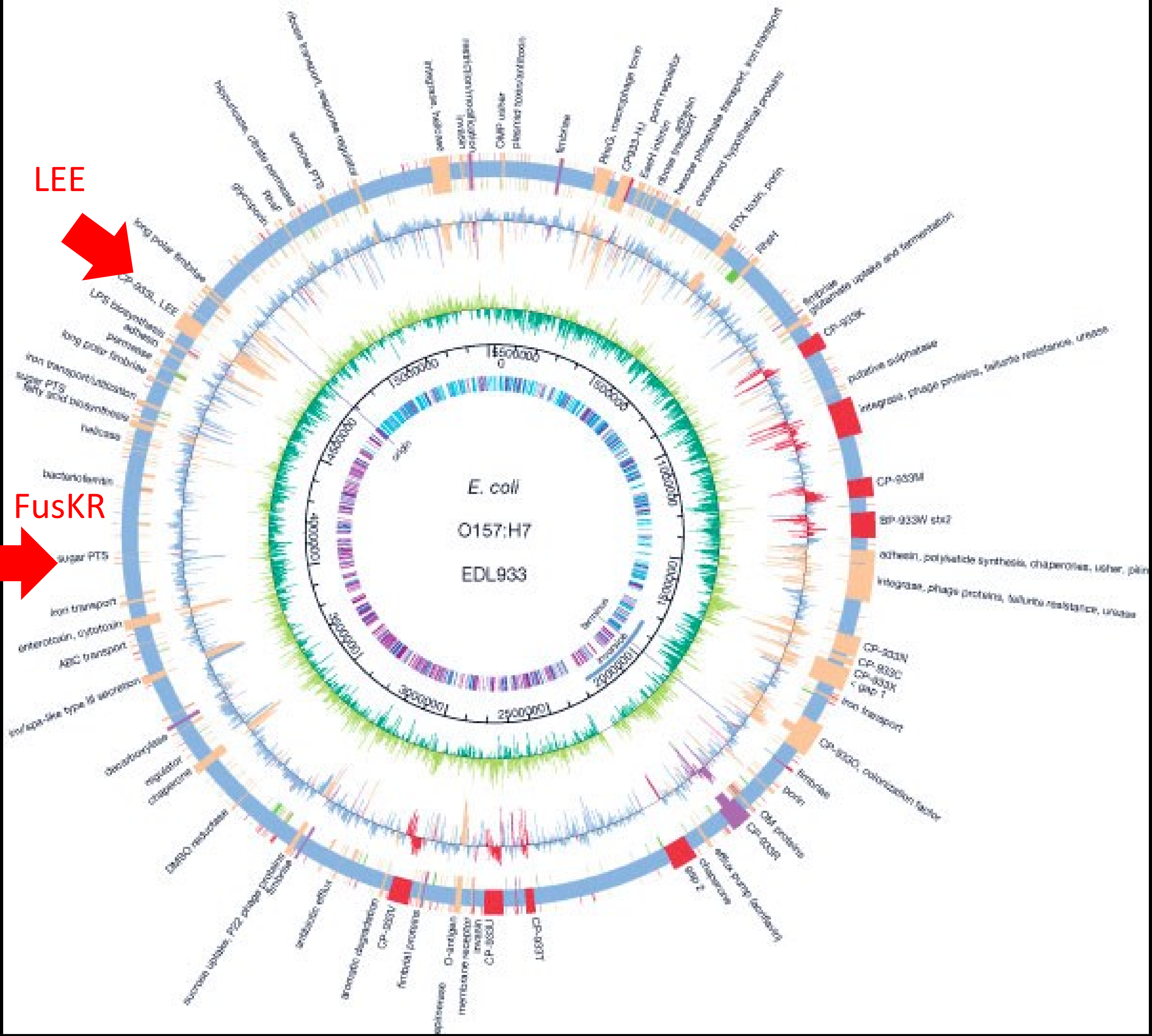
EHEC will exploit infochemicals from the microbiota and host







Bacteroides (*B. theta*) is very glycophagic it devotes 18% of its genomes to encode enzymes that cleave sugar from the mucus

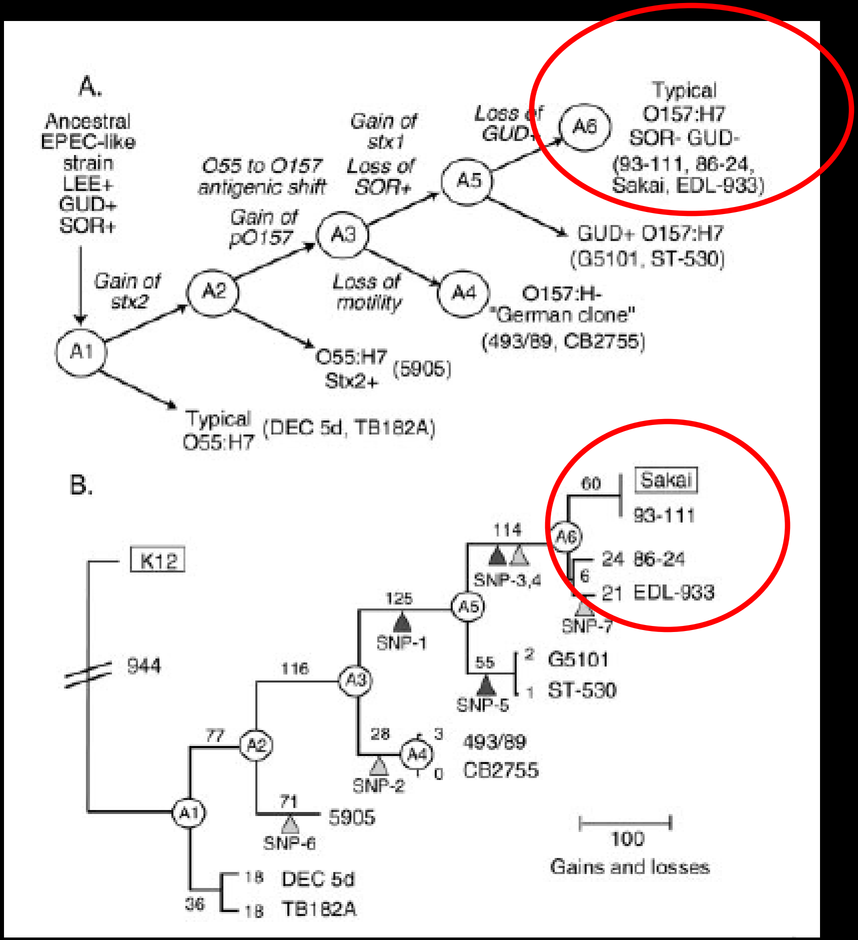


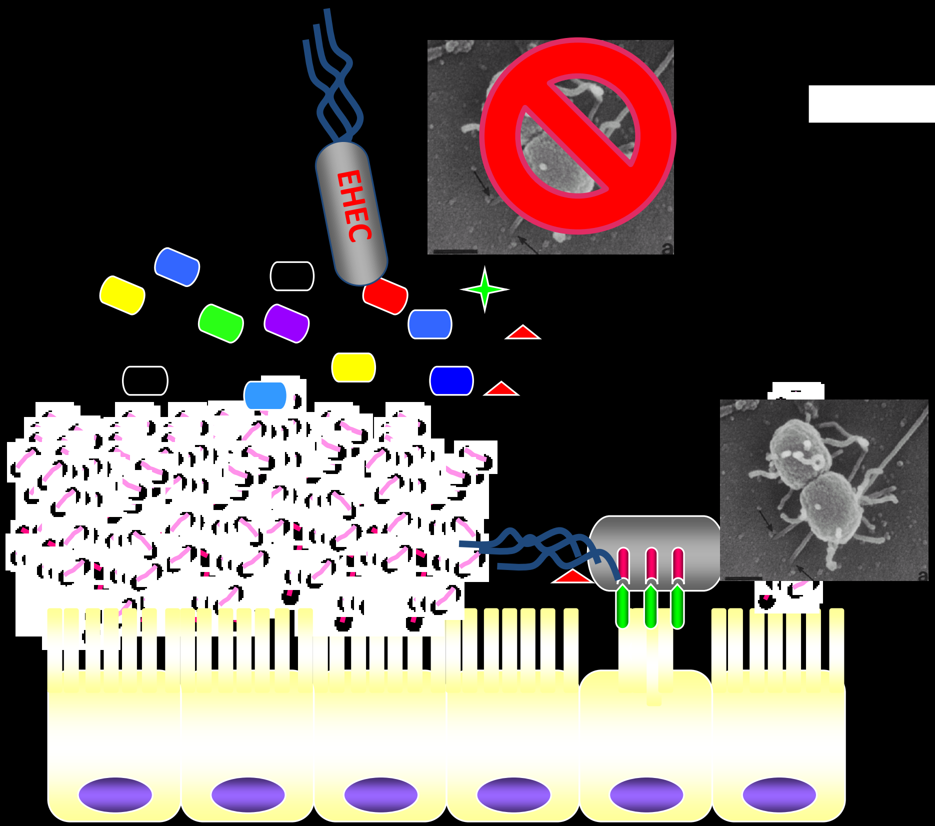
LEE

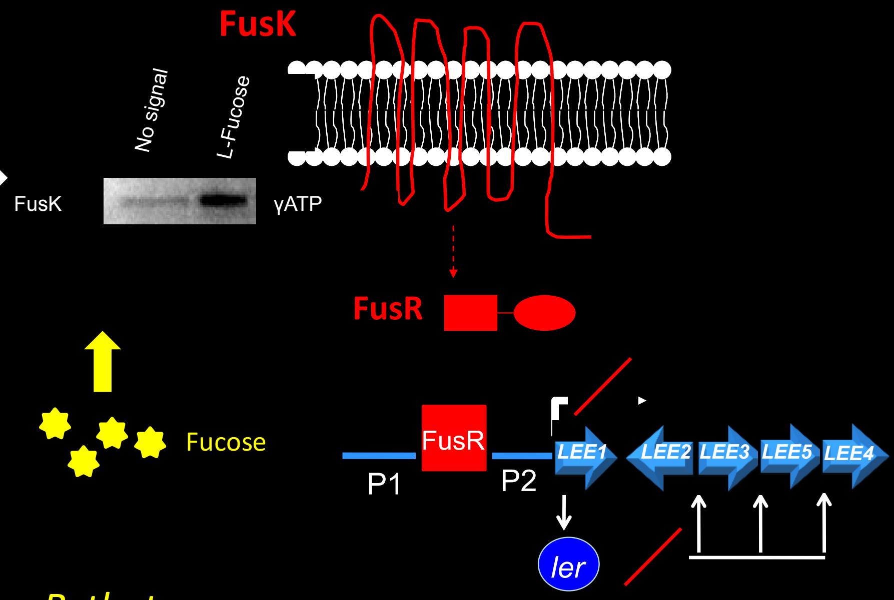
FusKR

Perna et al. Science 2001

Are pathogens the teenagers of the bacterial world (EHEC O157:H7 as an example)?

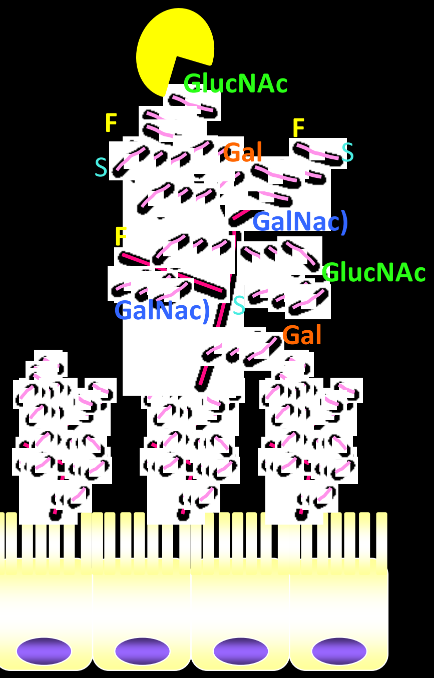






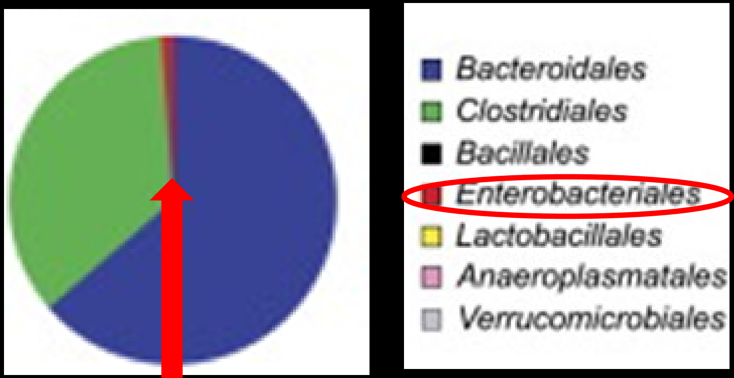
Represses the LEE

B. theta



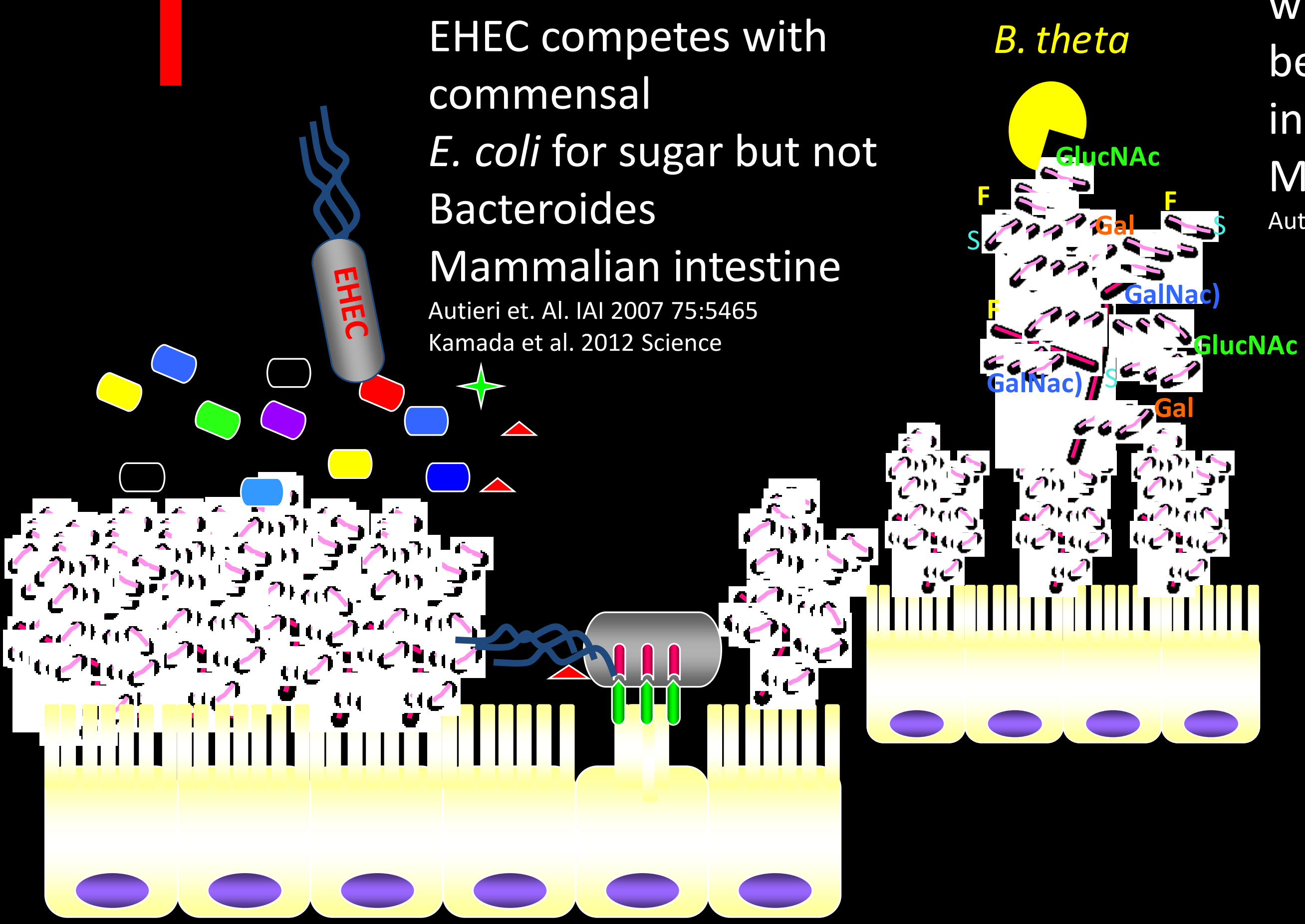
Alline Pacheco

Pacheco et al. Nature 2012



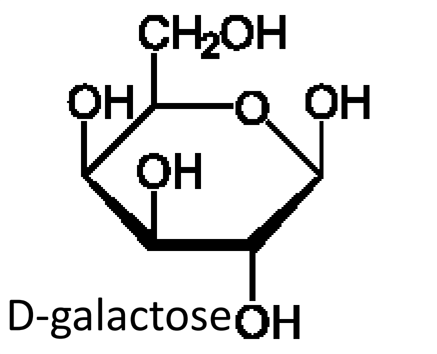
EHEC competes with commensal
E. coli for fucose, and commensal
E.coli use fucose better
EHEC prefers D-galactose, which it uses
better than commensal *E. coli* in the
Mammalian intestine

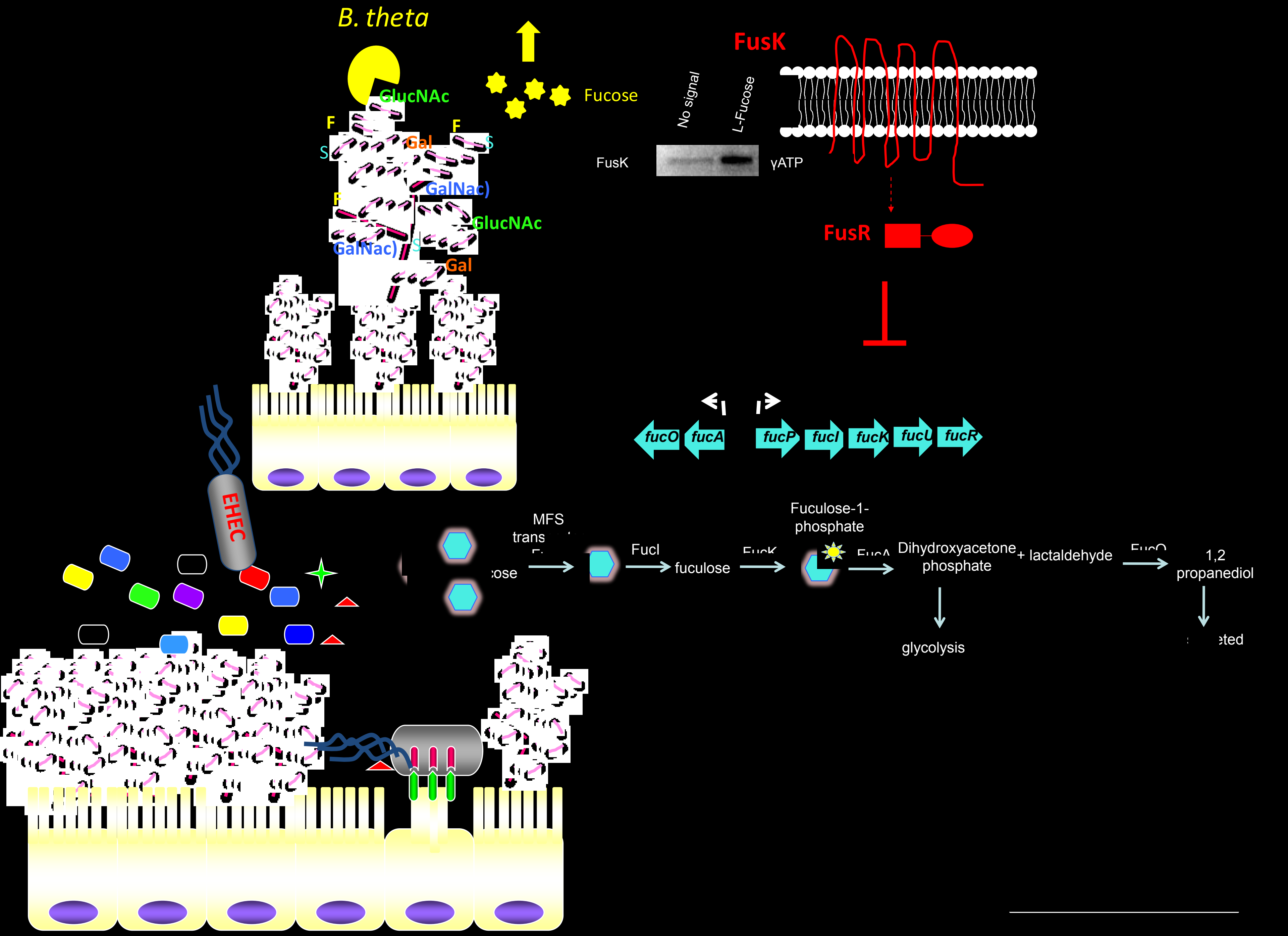
Autieri et. Al. IAI 2007 75:5465



EHEC competes with commensal
E. coli for sugar but not Bacteroides
Mammalian intestine

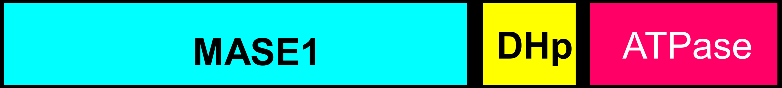
Autieri et. Al. IAI 2007 75:5465
Kamada et al. 2012 Science


D-galactose


B. theta
Fucose
GlucNAc
F
F
S
Gal
S
GalNac)
F
GlucNAc
GalNac)
S
Gal
EHEC
FusK
No signal
L-Fucose
FusK
γATP
FusR
fucO
fucA
fucP
fucI
fucK
fucU
fucR
MFS
trans
ose
FucI
fuculose
FucK
Fuculose-1-
phosphate
FucA
Dihydroxyacetone
phosphate
+ lactaldehyde
FucO
1,2
propanediol
glycolysis
eted

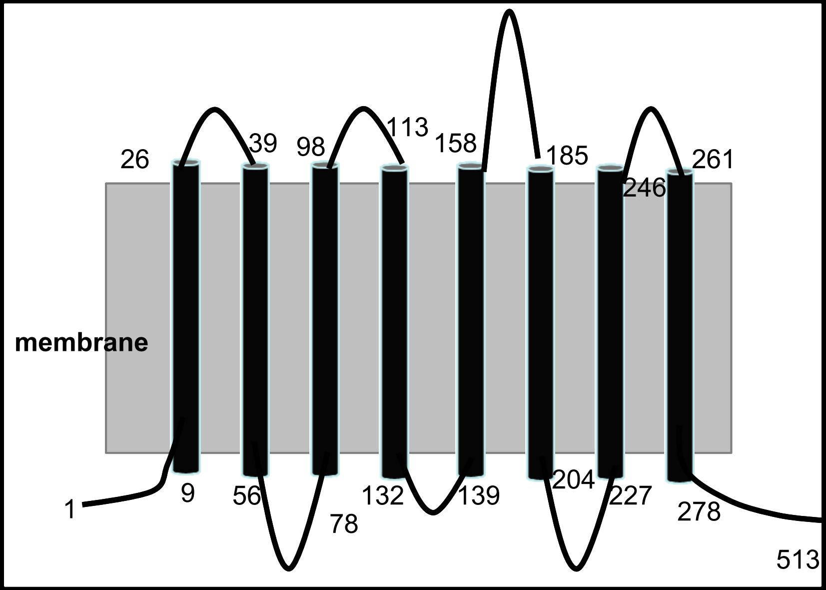
FusK and FusR domains

fusK = Fucose utilization sensor kinase



513 aa

57.8KDa



fusR = Fucose utilization regulator

209 aa

22.8KDa

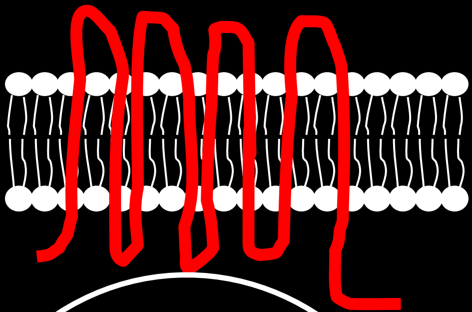
Search for sequence similarity (BLAST):

- EHEC O157:H7
- EPEC O55:H7
- *E.coli* ED1a
- *Citrobacter rodentium*

FusKR

- Two component system only present in EHEC, absent In all other E. coli
- FusKR represses LEE expression
- FusKR repress virulence expression
- FusKR repress the fucose utilization genes

What is FusK sensing?



But Vanessa FusK has 8 Transmembrane domans!

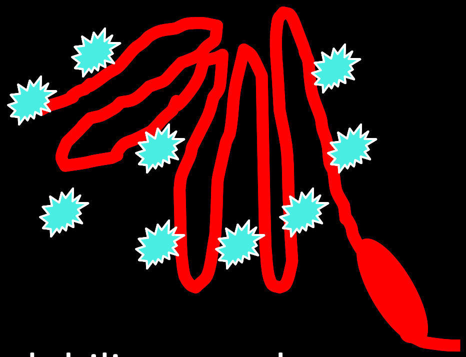
Alline Pacheco

Because I am a free B… baby

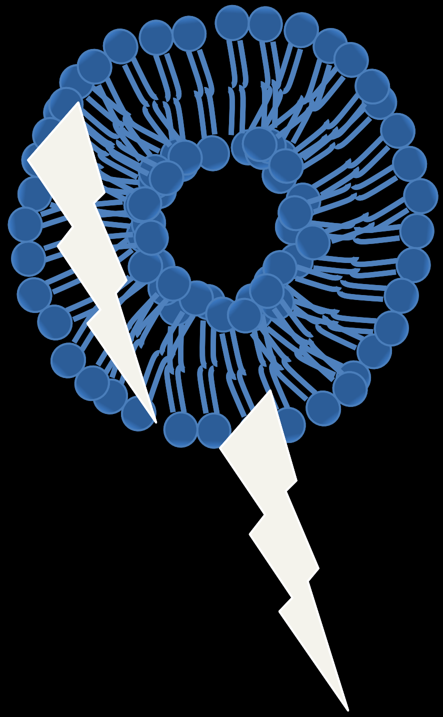
Too bad you got a Bad Project!

If you work for me
I am not easy to please…

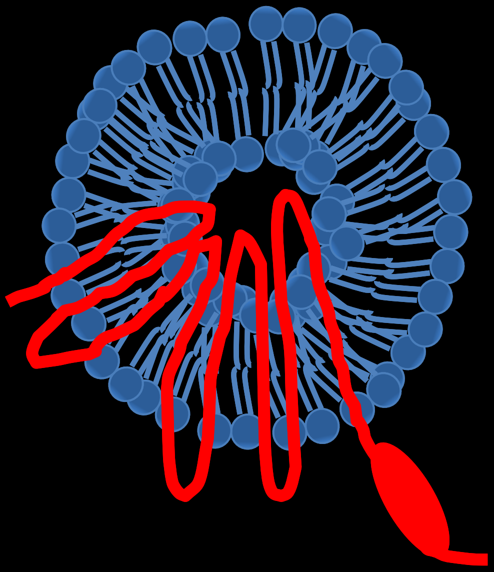
FusK purification and reconstitution into liposomes



Solubilize membrane-bound kinase with
1% Deoxycholate

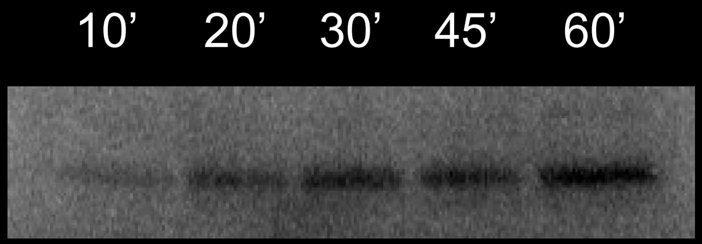


Freeze N2/ thaw RT (3X)
Let sit 1hour RT



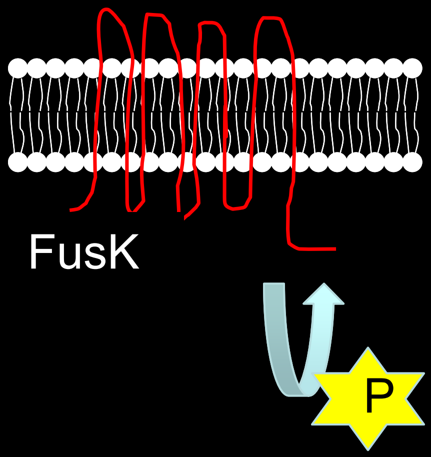
FusK in a functional HK *in vitro*

FusK autophosphorylates when reconstituted into liposomes

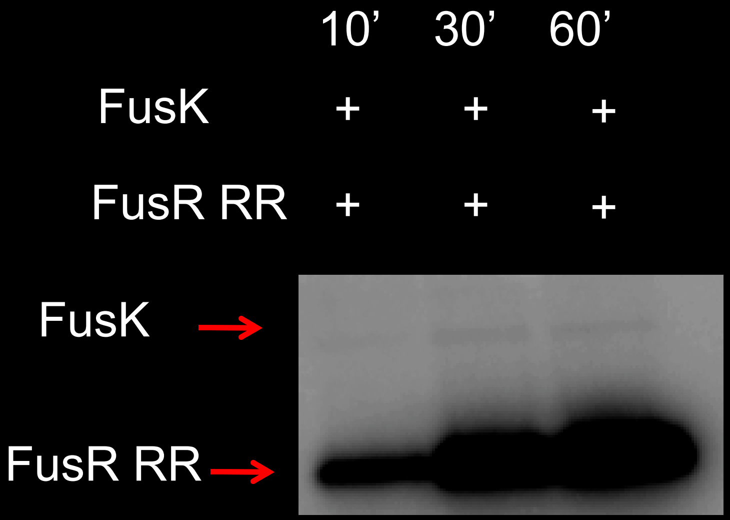


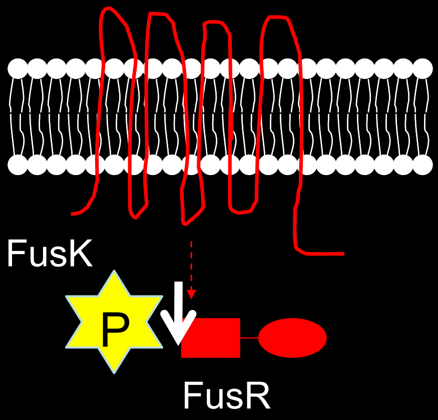
FusK

(4.3uM kinase)

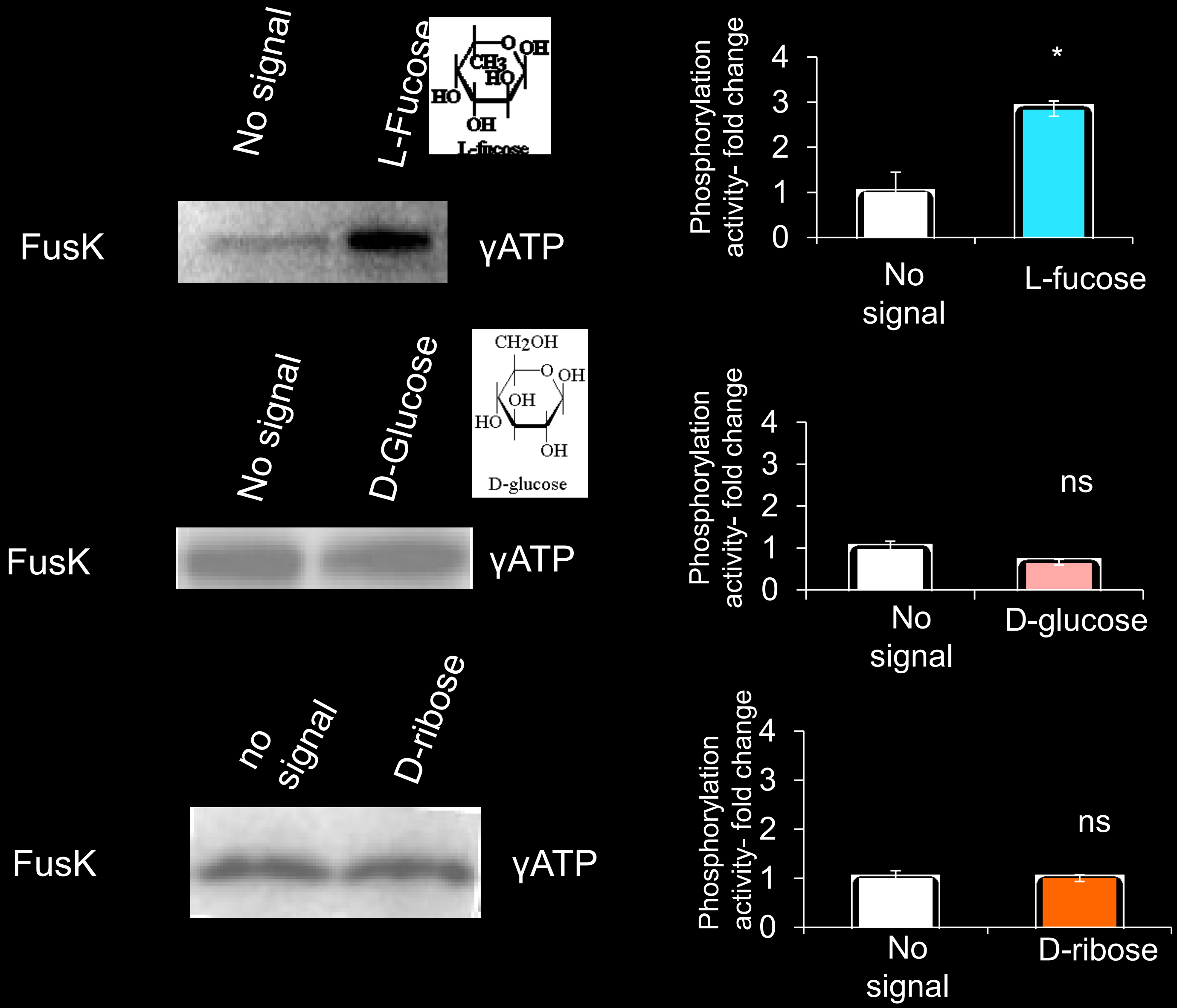


FusK HK and FusR RR are a cognate pair TCS

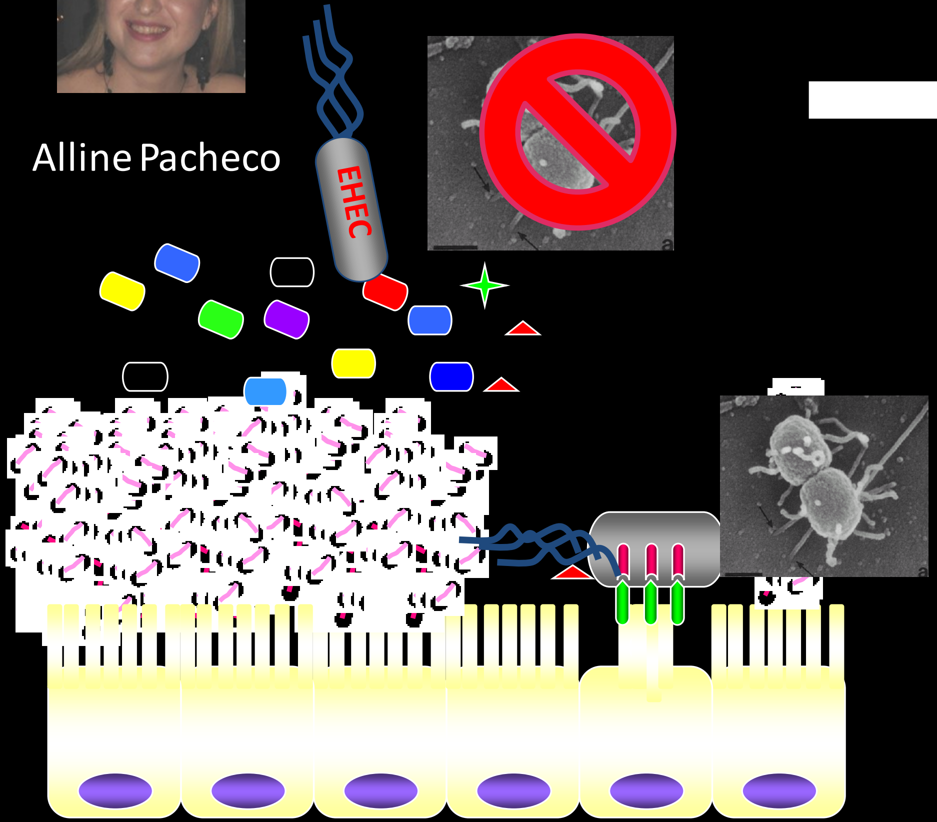




FusK autophosphorylates upon sensing L- fucose

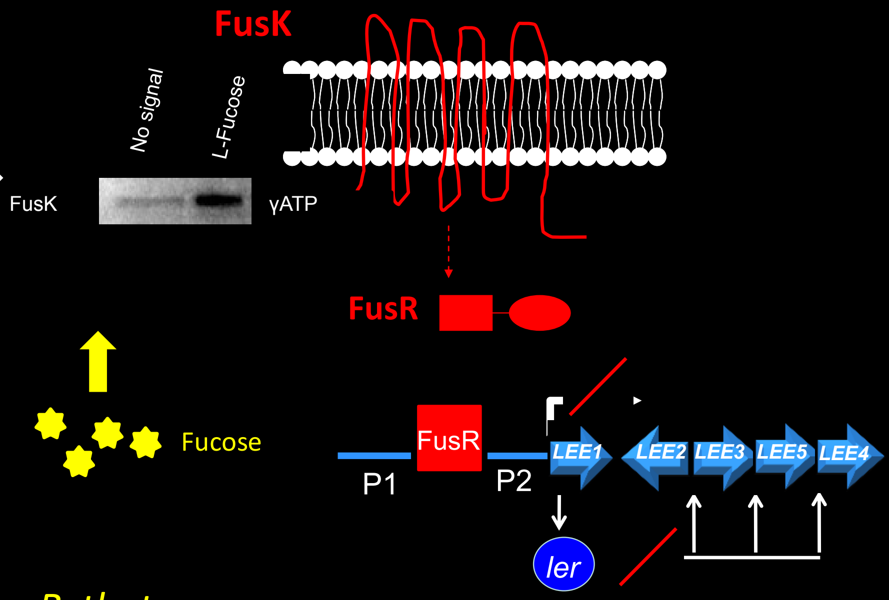


Alline Pacheco

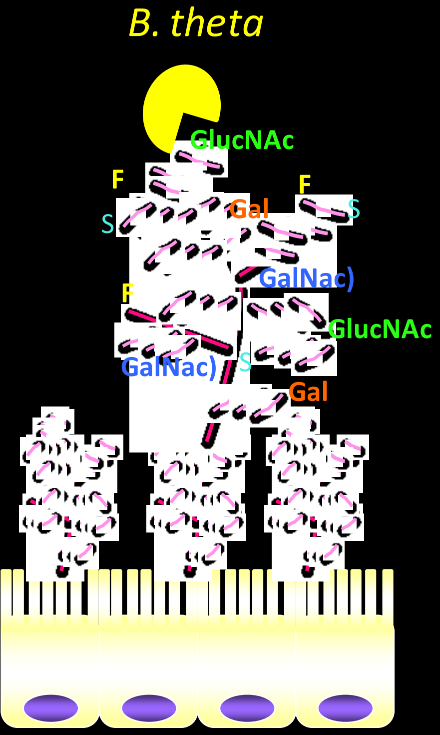


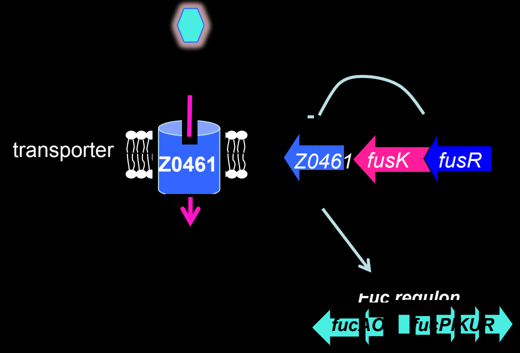
EHEC competes with commensal *E. coli* for fucose, and commensal *E.coli* use fucose better
EHEC prefers D-galactose, which it uses better than commensal *E. coli* in the Mammalian intestine

Autieri et. Al. IAI 2007 75:5465



Represses the LEE





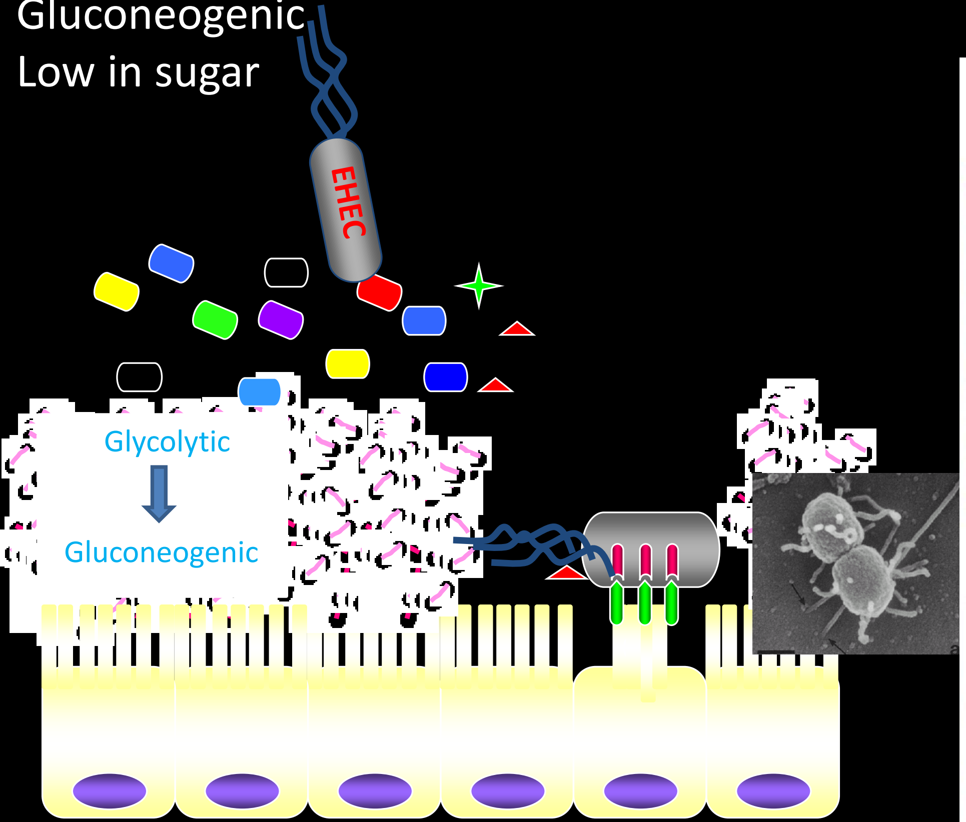
Repress Fucose utilization

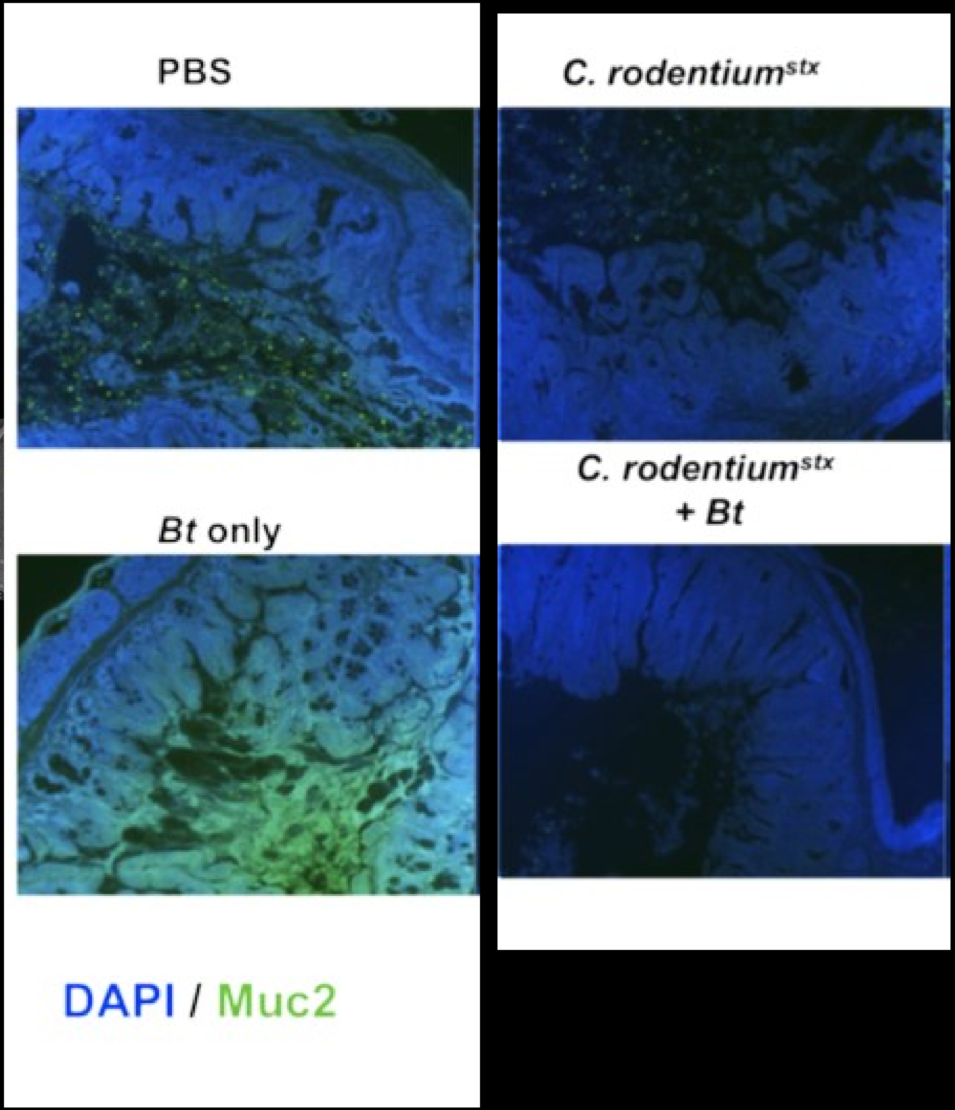
Pacheco et al. Nature 2012, 492:113

Glycolytic
lots of sugar

Gluconeogenic
Low in sugar

EHEC produces mucinases that are enzymes that destroy the mucus layer



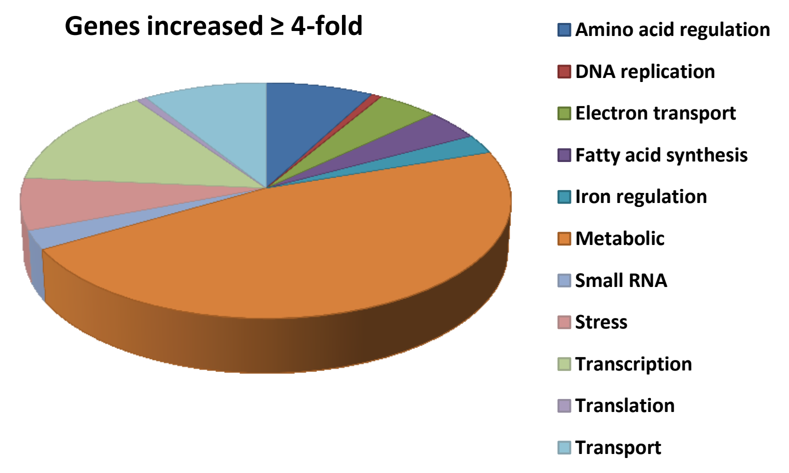


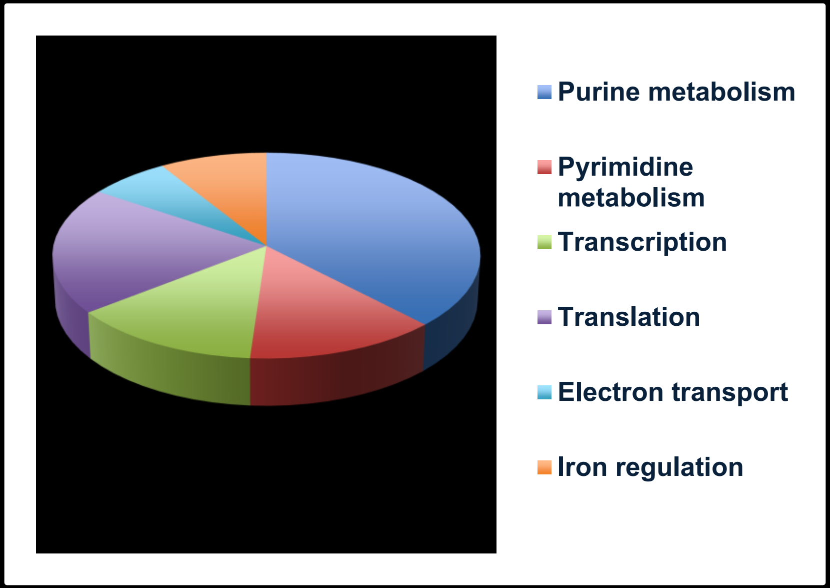
Meredith Curtis

Approximately 20% of the *E. coli* probes have increased expression in the presence of *B. theta*

Meredith Curtis

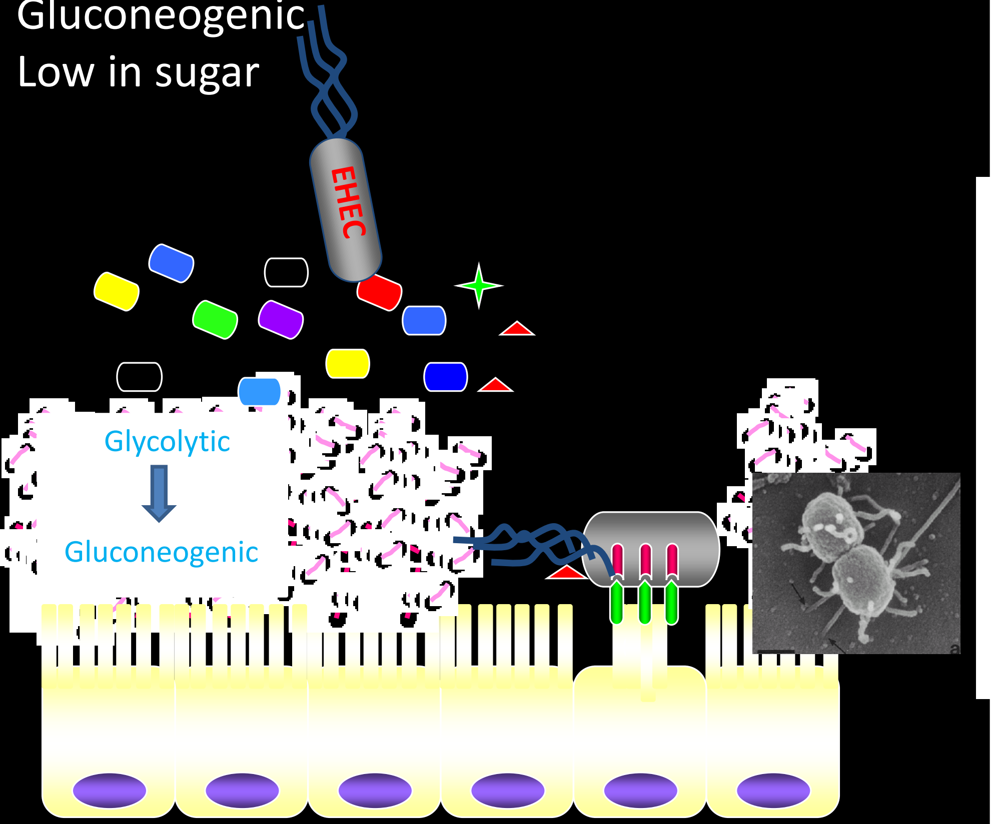
	MG1655	EDL933	Sakai	CFT073	IG	Control	Total
Increase	**886**	**579**	**107**	**326**	**424**	**32**	**2354**
Marginal increase	**27**	**25**	**10**	**30**	**26**	**0**	**118**
No change	**2113**	**948**	**229**	**2054**	**797**	**149**	**6290**
Marginal decrease	**990**	**225**	**27**	**67**	**46**	**13**	**1368**
Decrease	**54**	**10**	**0**	**9**	**4**	**1**	**78**
Total	**4070**	**1787**	**373**	**2486**	**1297**	**195**	**10208**

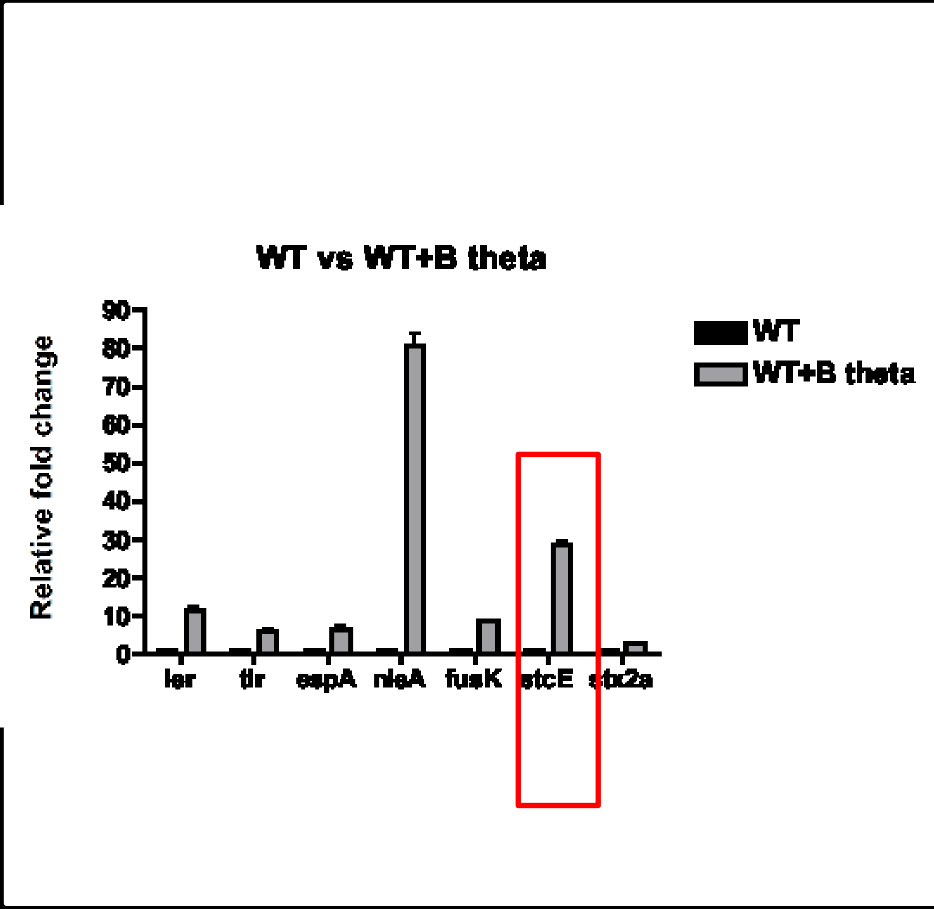




Glycolytic
lots of sugar

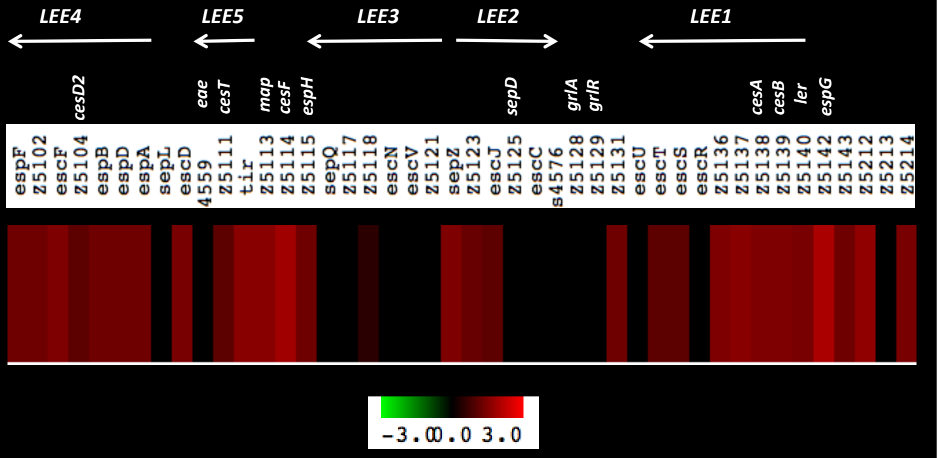
Gluconeogenic
Low in sugar

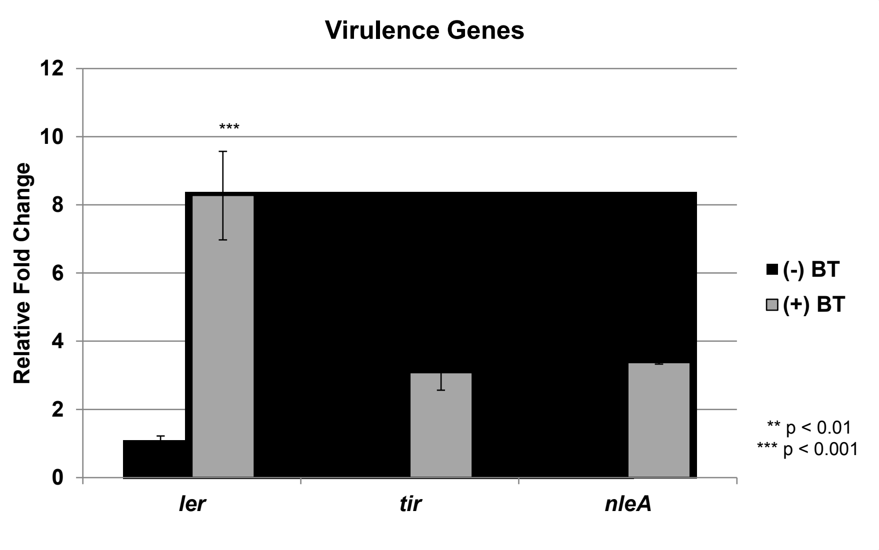


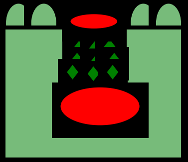


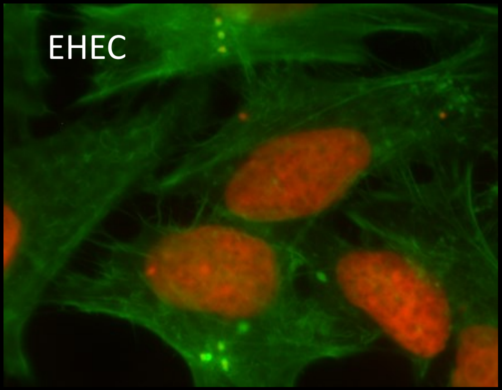
Meredith Curtis

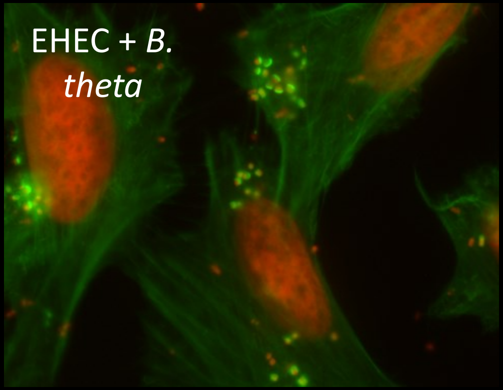
Expression of the LEE is increased in the presence of *B. theta*

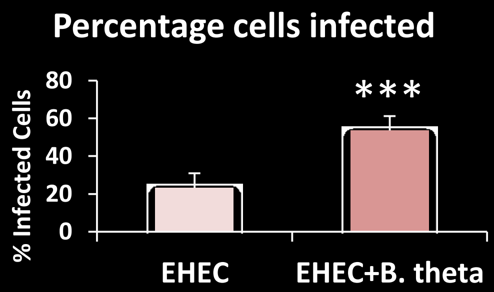




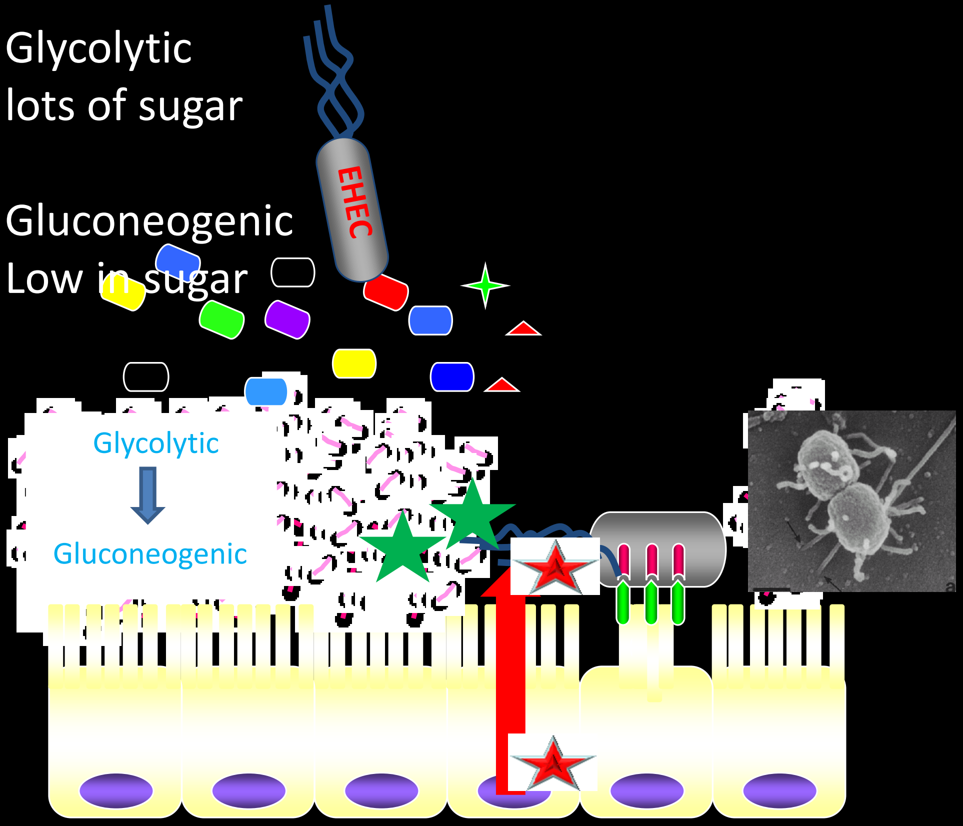




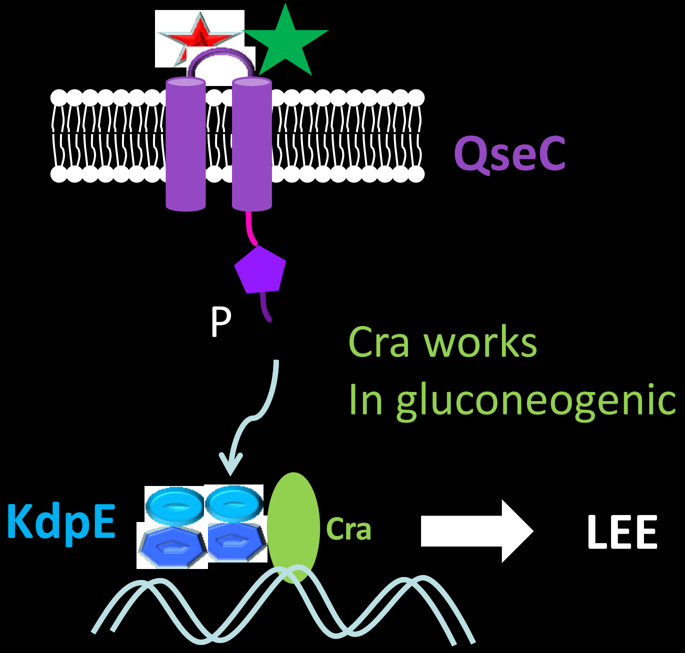




*** p<0.001



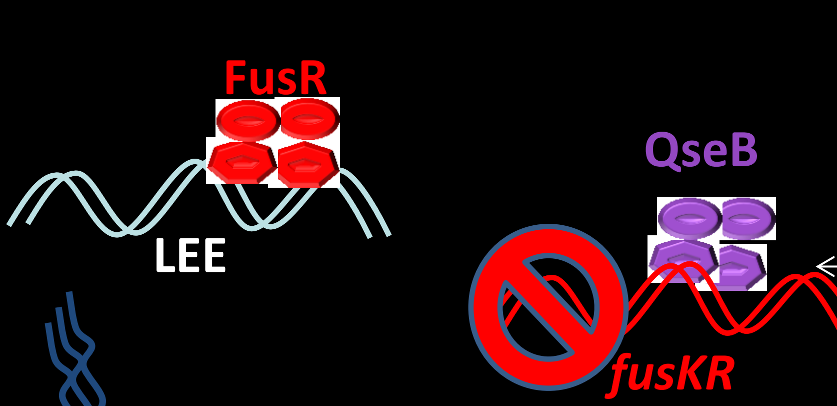
Norepinephrine/epineprine

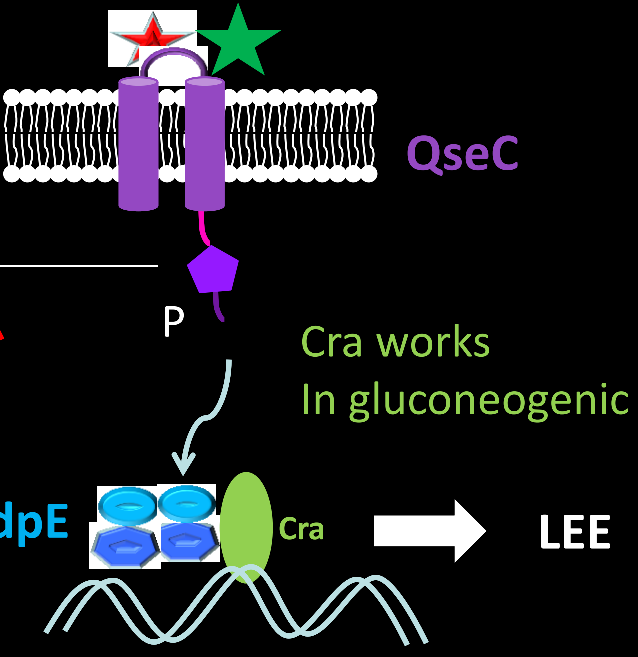


Jacky
Njoroge

Under gluconeogenic conditions
B.theta, E. coli and EHEC itself
Makes the AI-3 signal

Njoroge et al. mBio 2012, 3:00280; Hughes et al. Plos Pathogens 2009; Rasko et al. Science 2008
Clarke et al. 2006; Sperandio et al. 2003



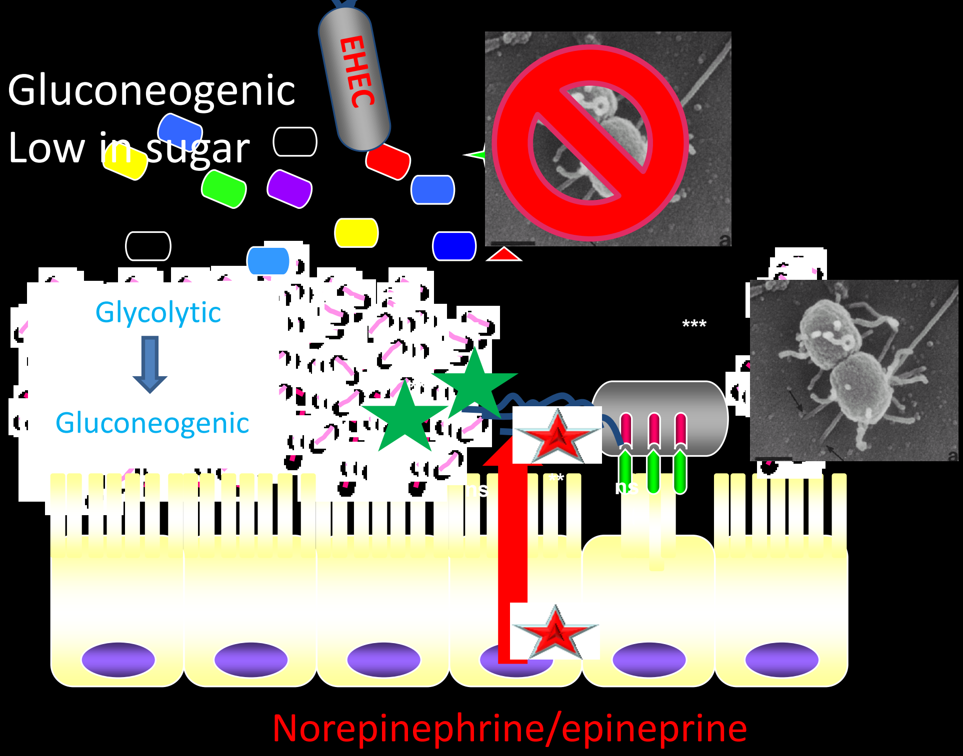


Cra works
In gluconeogenic

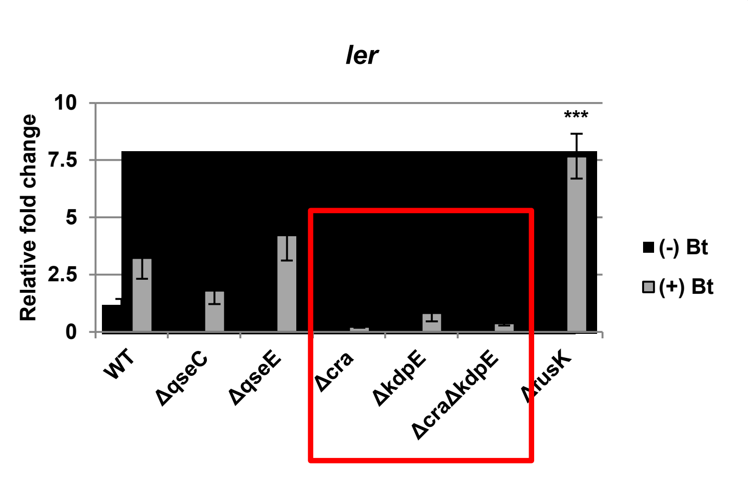
KdpE

Glycolytic
lots of sugar

Gluconeogenic
Low in sugar



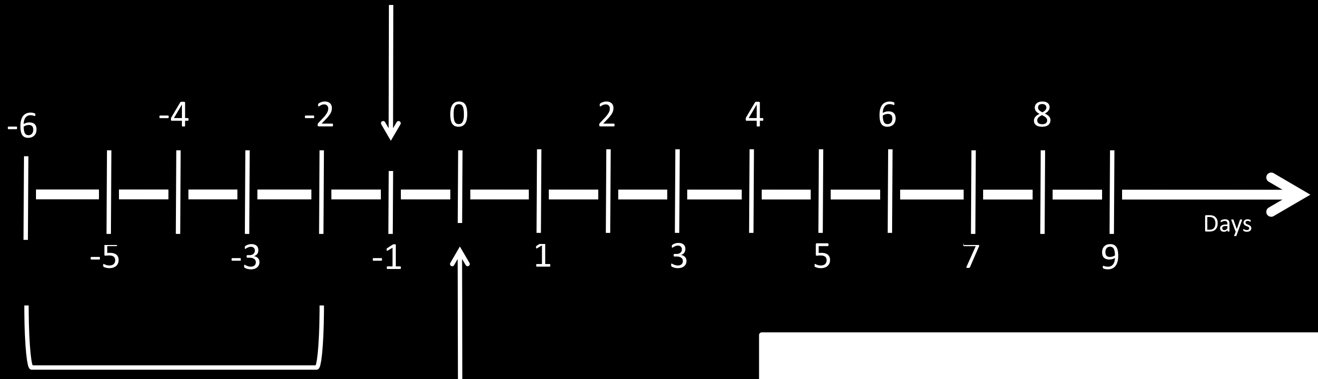
Norepinephrine/epineprine



Under gluconeogenic conditions *B.theta, E. coli* and EHEC itself makes the AI-3 signal
And lots of succinate, with Low amounts of fucose

What does that mean to me, a mammal???

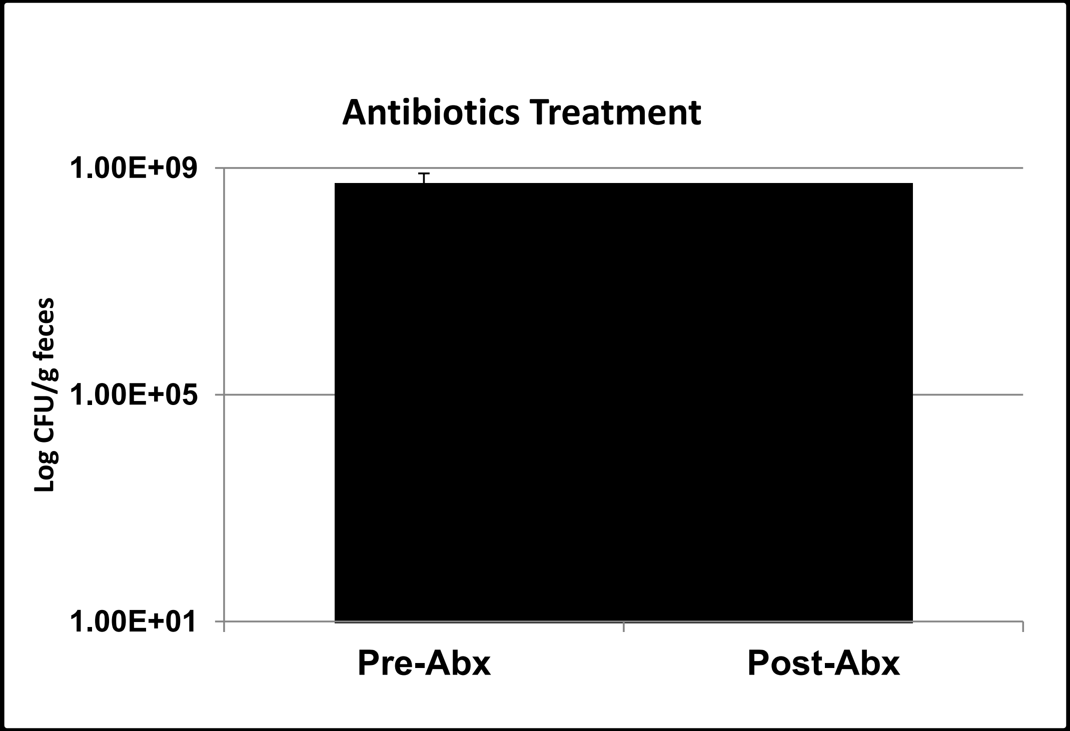
Is *C.rodentium* EHEC more virulent *in vivo* when *B. theta* is present?



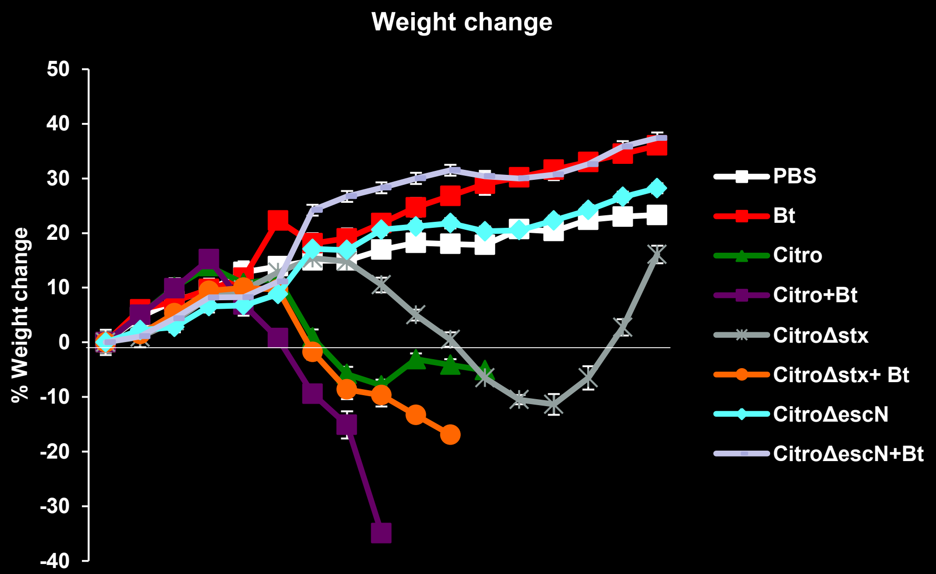
Inoculate C3H/HeJ mice with 3×10^9 CFU *B. theta*

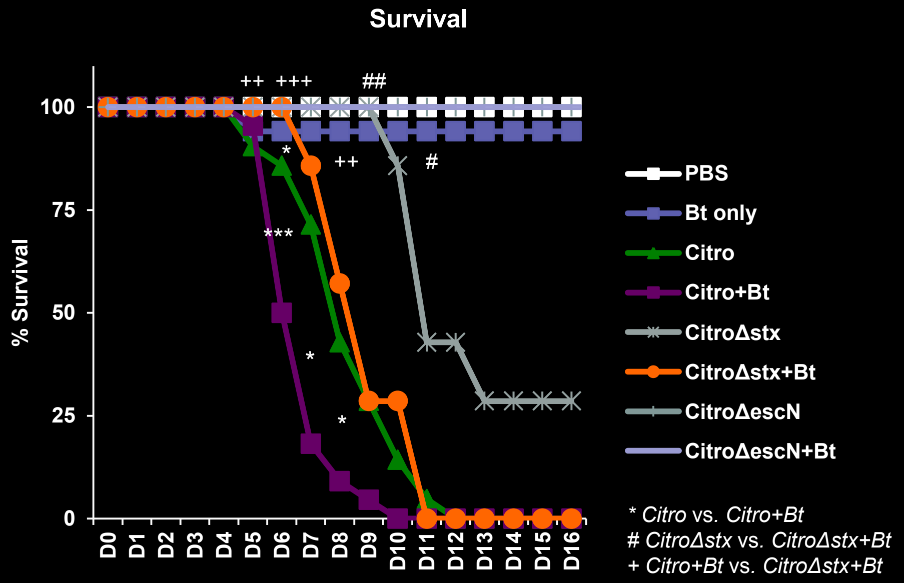
Antibiotics treatment (Cocktail of ampicillin, neomycin, metronidazole, vancomycin)

Infect with 10^9 CFU *Citro*

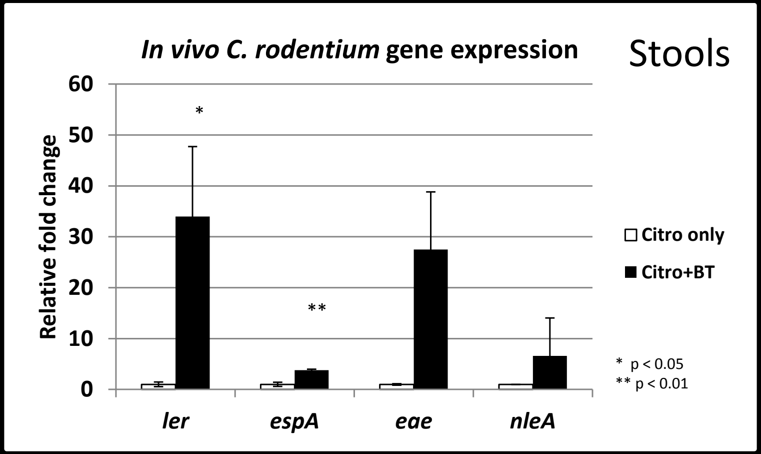


C.rodentium (EHEC) is more virulent in the presence of *B. theta*



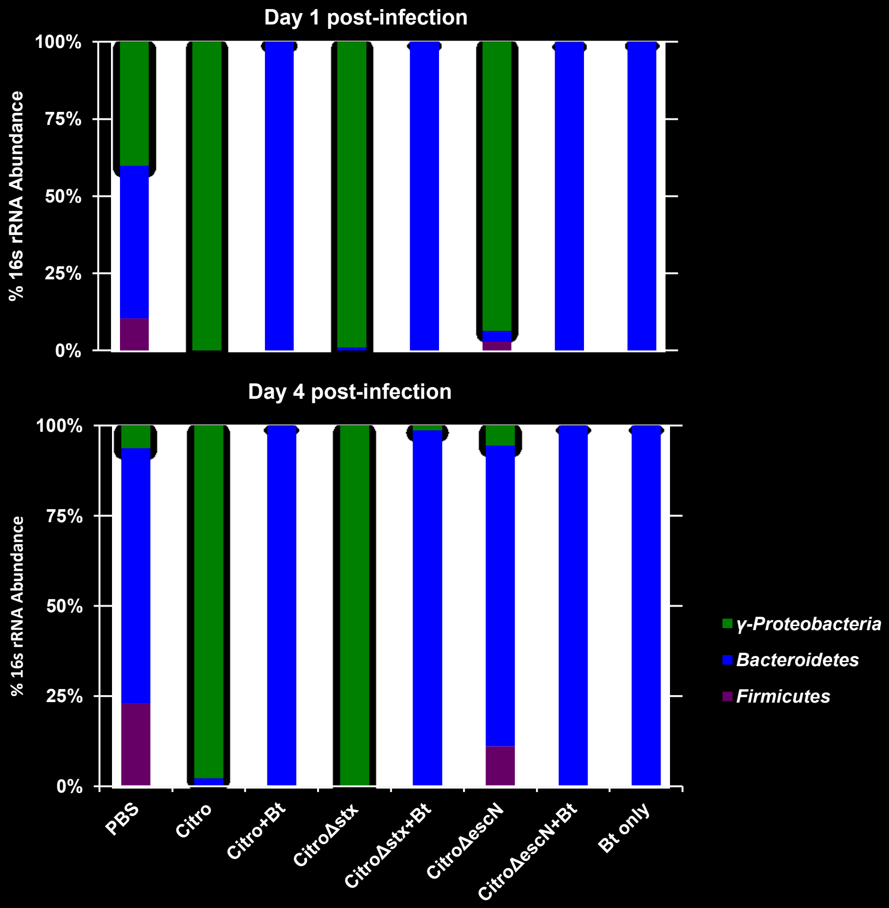


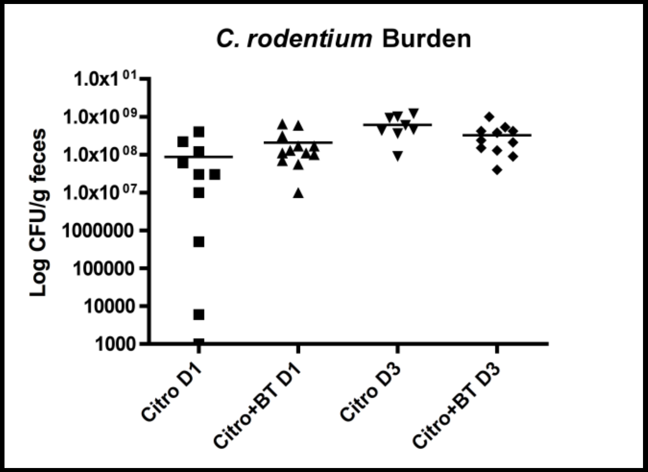
* *Citro* vs. *Citro+Bt*
\# *CitroΔstx* vs. *CitroΔstx+Bt*
\+ *Citro+Bt* vs. *CitroΔstx+Bt*

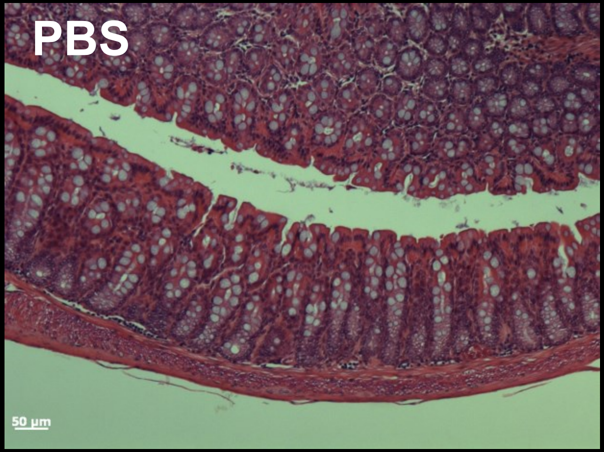


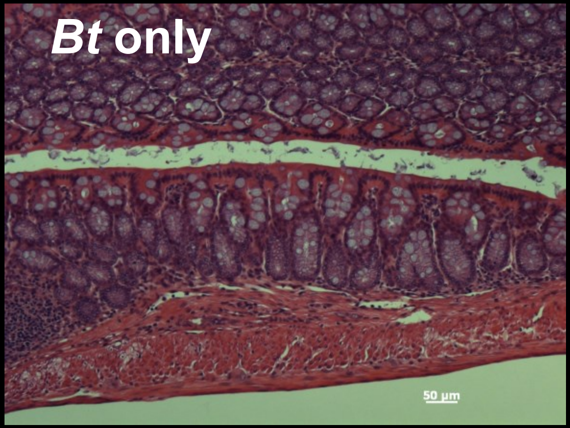
* $p < 0.05$
** $p < 0.01$

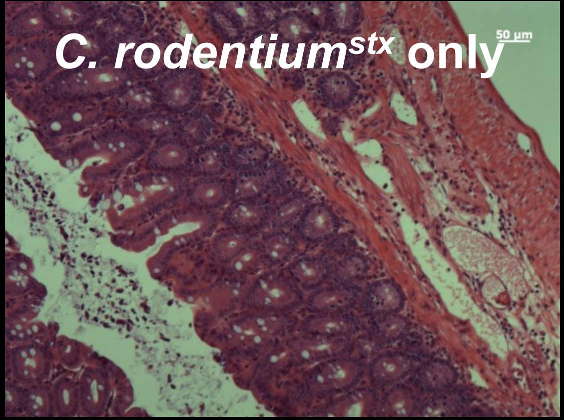
Microbiota phyla composition

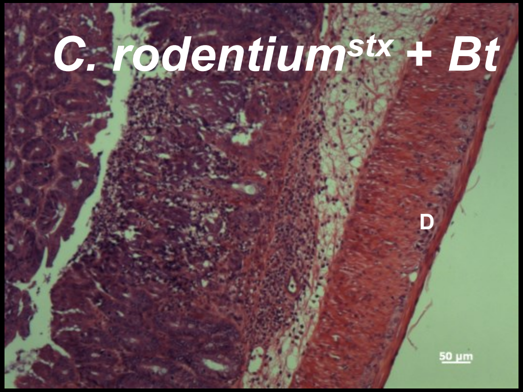


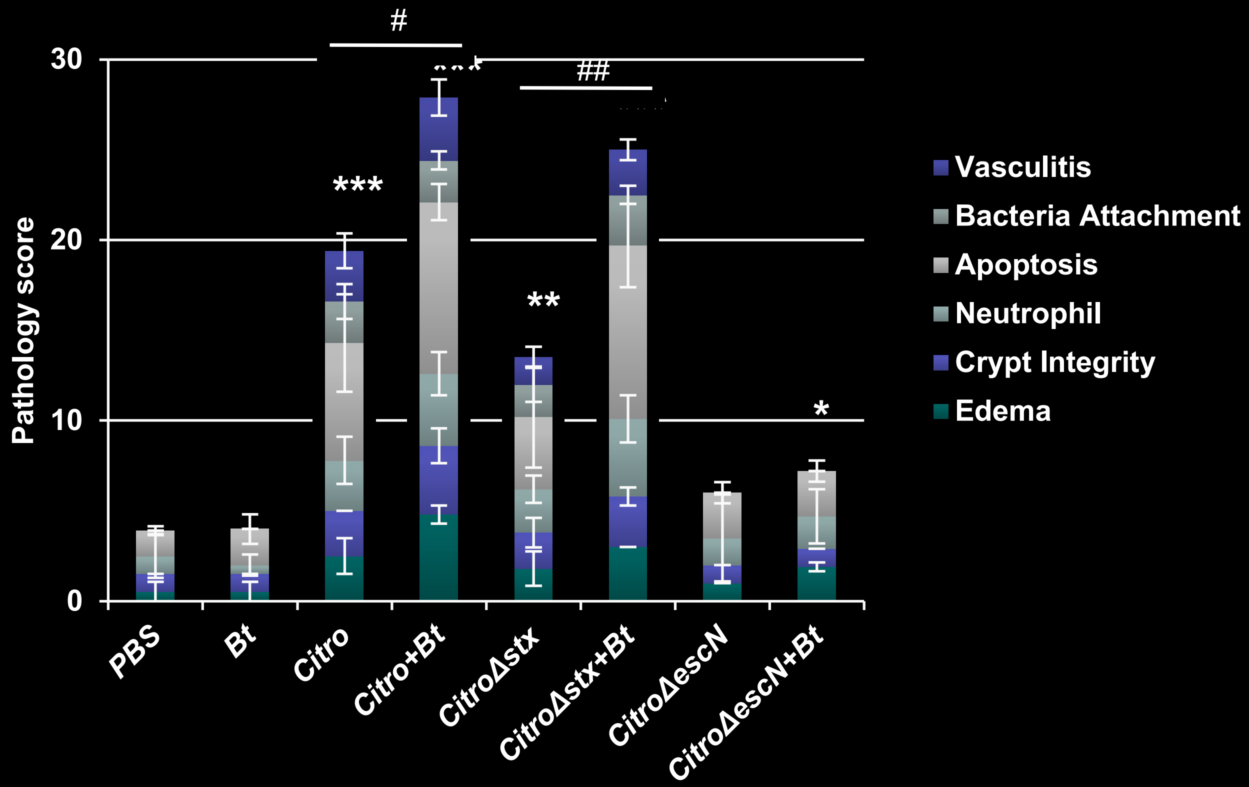



PBS
50 µm


Bt only
50 µm


C. rodentium^{stx} only
50 µm

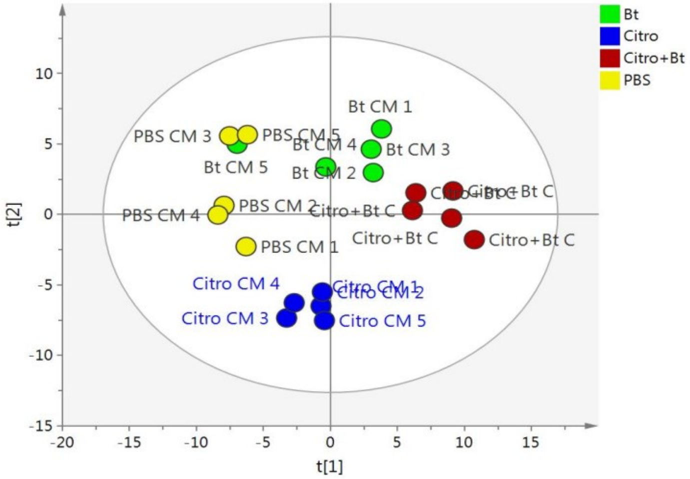

C. rodentium^{stx} + *Bt*
D
50 µm


Pathology score
0
10
20
30
#
##
***
**
*
PBS
Bt
Citro
Citro+Bt
CitroΔstx
CitroΔstx+Bt
CitroΔescN
CitroΔescN+Bt
Vasculitis
Bacteria Attachment
Apoptosis
Neutrophil
Crypt Integrity
Edema

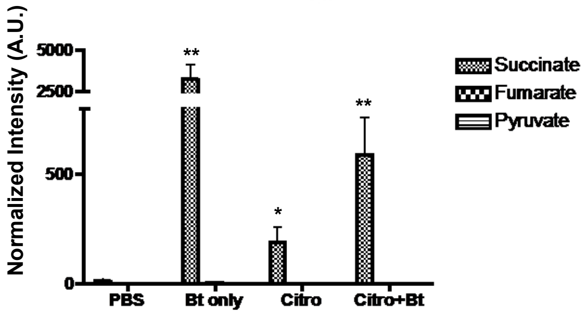
A

Metabolite	Bt/PBS	Citro/PBS	Citro+Bt/PBS	Citro+Bt/Citro	Citro+Bt/Bt
2-hydroxyglutaric acid	38.66	0.90	229.60	256.23	5.94
CMP	1.07	1.08	3.65	3.39	3.40
cytidine	0.65	0.06	0.40	6.16	0.61
gluconic acid	0.66	0.22	0.09	0.41	0.14
guanosine	0.42	0.08	0.26	3.09	0.62
2-deoxycytidine	0.73	0.05	0.27	5.96	0.37
2-isopropylmalic acid	1.42	2.44	0.66	0.27	0.46
2-ketohaxonic acid	7.89	1.47	4.71	3.21	0.60
3-hydroxybutyrate	2.12	1.13	2.36	2.09	1.11
7-methylguanosine	5.14	0.87	3.29	3.76	0.64
adenosine	5.18	1.86	5.62	3.02	1.09
ascorbic acid	1.35	2.38	0.63	0.26	0.47
cadaverine	1.72	5.73	9.39	1.64	5.47
carnitine-C3	3.18	0.96	8.85	9.19	2.78
cysteine	282.13	155.28	565.61	3.64	2.00
dAMP	0.80	0.80	5.29	6.63	6.58
deoxyinosine	0.51	0.03	0.15	4.93	0.30
deoxyuridine	0.36	0.00	0.10	58.50	0.29
flavin adenine dinucleotide	3.13	1.30	3.55	2.72	1.13
glucarate	0.79	0.25	0.61	2.40	0.77
glutamate/NMDA	2.05	2.29	5.15	2.25	2.51
glycerate	48.54	0.90	72.39	80.78	1.49
glycerophosphorylcholine	1.29	0.22	0.14	0.62	0.10
glycine	0.71	2.56	2.44	0.95	3.42
GSH	82.25	0.90	3463.48	3865.10	42.11
guanine	0.60	0.13	0.37	2.97	0.62
hydroxyisocaproic acid	7232.29	1906.05	1989.32	1.04	0.28
kynurenine	0.84	1.21	3.34	2.76	3.98
malic acid	6.44	3.84	11.93	3.10	1.85
NAD	8.51	2.77	22.03	7.96	2.59
nicotinamide	0.50	0.54	1.43	2.66	2.86
ornithine	8.12	7.25	5.93	0.82	0.73
pantothenic acid	2.15	1.80	3.84	2.13	1.78
phenyl-lactic acid	236.01	139.47	401.03	2.88	1.70
S-adenosyl-homocysteine	131.79	0.90	90.79	101.32	0.69
succinate/methyl-malonic acid	200.57	37.06	201.05	5.43	1.00
thiamine	0.37	1.07	0.07	0.07	0.20
thymidine	0.57	0.03	0.23	6.59	0.39
UDP	1.04	5.52	15.91	2.88	15.26
uridine	0.58	0.15	0.45	3.10	0.79

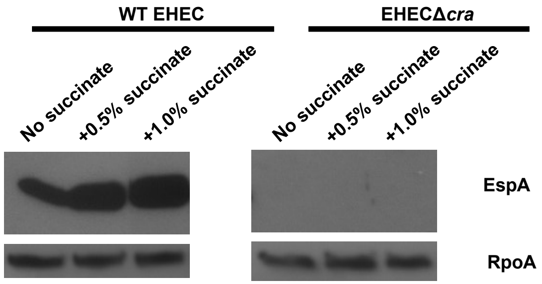
B



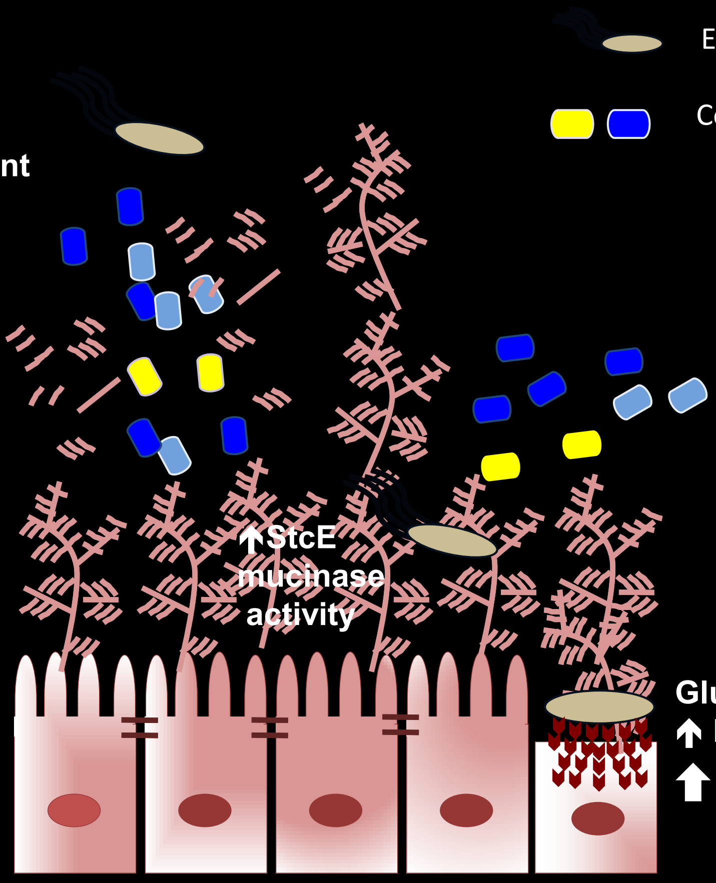
C



D



Model: Nutritional cues regulate LEE expression



EHEC

Commensal bacteria

Glycolytic environment
↓ LEE, ↑ FusK

Lumen

Mucus layer

↑StcE mucinase activity

Intestinal epithelium

Gluconeogenic environment
↑ LEE, ↓ FusK

↑ QseC/KdpE/Cra

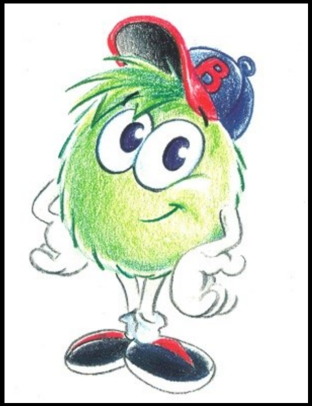
Summary

B. theta increases virulence gene expression in EHEC and *C. rodentium*

C. rodentium murine mediated disease is increased in the presence of *B. theta*

Although the microbiota is usually regarded as a protective barrier to enteric Infections, it can be exploited by certain pathogens to promote and enhance their virulence


illustration: Don Smith



Sperandio LAB

Regan Russell
Elizabeth Cameron
Meredith Curtis
Juan Hernandez David
Reed Pifer
Y Nguyen
Kim Carlson Banning
Ben Knowles

Former members

Nicola Reading
David Hughes
Aline Pacheco
Jacqueline Njoroge
David Rasko
Marcie Clarke
Cristiano Moreira
Charley Gruber

Collaborators:

John Leong Tufts University Medical School
James Richardson UT Southwestern
Sanjeev Narayanan Kansas State University
Ralph Deberardinis UT Southwestern
Zeping Hu UT Southwestern

NIAID/ The Ellison Medical Foundation/ Burroughs Wellcome Fund