CRIS 2030-42000-050

Ecology and Detection of Human Pathogens in the Produce Production Continuum

WRRC, Albany, CA

Produce Safety and Microbiology RU

Lisa Gorski*, Maria T. Brandl, Michael B. Cooley, Michelle Q. Carter, Peng Tian

HuNoV-Related Research At PSM:

1. Develop assays to detect and estimate HuNoV infectivity
2. Develop system to express HuNoV capsid proteins on the bacterial surface, and isolation of candidate ligands for HuNoV binding
3. Characterization of binding and release conditions for bacteria-human norovirus capsid P protein complex (BPC) from romaine lettuce extract
4. Characterization of bacterial/viral interaction

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Development of novel assays for the detection of HuNoV and estimation of HuNoV infectivity

- *In Situ* Capture RT-qPCR
- *In Situ* Aptamer RT-qPCR
- Redesigned RT-qPCR
- Digital RT-qPCR

1. *Wang, D.,* and **Tian, P**. (2014). Inactivation Conditions for Human Norovirus Measured by an *In Situ* capture-qRT-PCR Method. Int. J. Food Microbiol., 72:76-82
2. *Wang, D.*, Xu, S, Yang, D., Young, G. H., and **Tian, P.** (2014). A new *In Situ* Capture-qRT-PCR method uses for an alternative approach to determine inactivation of Tulane virus. Appl. Env. Microbiol., 80: 2120-2124
3. **Tian, P., Yang, D., Shan, L.,** Wang, D., Li, QGorski, L., Lee, B., Quinones, B., and Cooley, M. (2017). Concurrent Detection of Human Norovirus and Bacterial Pathogens in Water Samples from an Agricultural Region in Central California Coast. **Front. Microbiol.** 8:1560.
4. Zhou, Z, Tian, Z, Li, Q, **Tian, P**., Wu, Q., Wang, D., and Shi, X. (2017) *In Situ* Capture RT-qPCR: A New Simple and Sensitive Method to Detect Human Norovirus in Oysters. **Front. Microbiol**. 8:554
5. **Tian, P.,** Yang, D., Lei, S., Li, Q., Liu, D., Wang, D., Estimation of human norovirus infectivity from environmental water samples by In Situ Capture RT-qPCR method. (2017). **Food and Enviro. Virol**. 10”29-38.
6. Liu., D., Zhang, Z., Yin, Y., Jia, F., Wu, Q., **Tian., P**., Wang, D. (2019) Development and evaluation a novel *in situ* target-capture approach for aptamer selection of human noroviruses . Talanta 193:199-205
7. Liu., D., Zhang, Z., Wu, Q., **Tian., P.,** Geng, H., Wang, D. Re-digned RT-qPCR for detection of GI and GII human noroviruses Food Science and Engineering Submitted.

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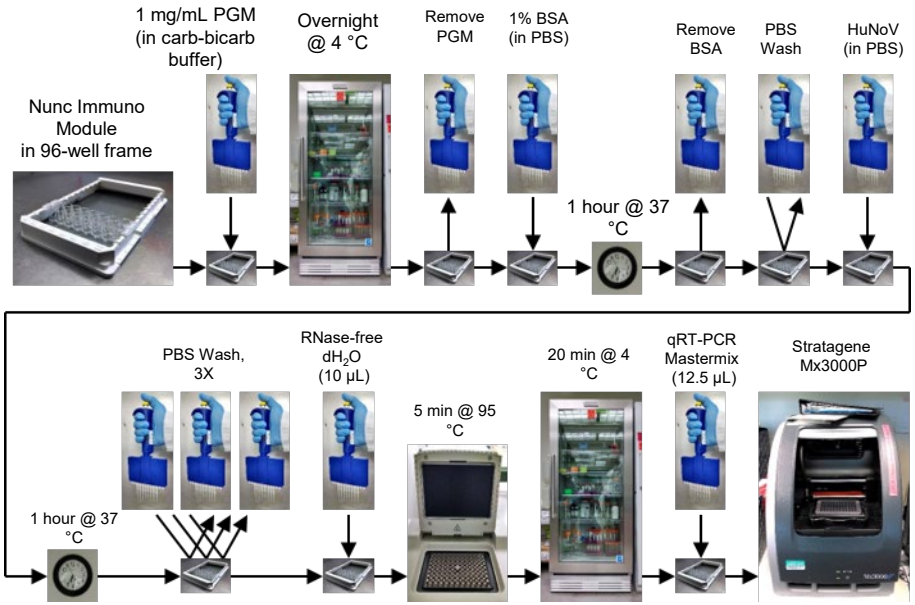
In Situ Capture RT-qPCR (ISC-RT-qPCR) Method to Detect Infectious HuNoV



Method

1. Wells coated with pig stomach mucin (receptors for HuNoV) → Virus capture
2. Rinses remove inhibitors
3. Heated to release RNA → Detection by qRT-PCR

Benefits:

- Better indicator of infectivity (only encapsulated RNA is detected)
- Concentrates HuNoV and removes inhibitors
- RNA extraction or transfer of virus from magnetic beads not needed.
- Adaptable to high throughput systems

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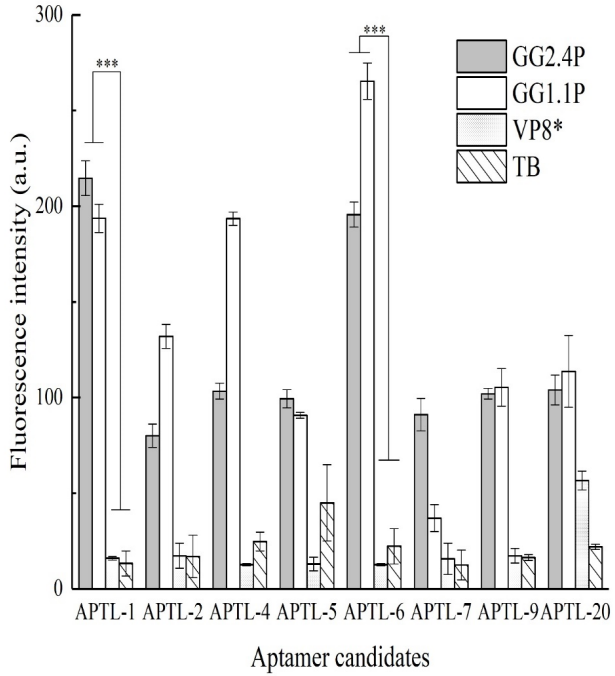
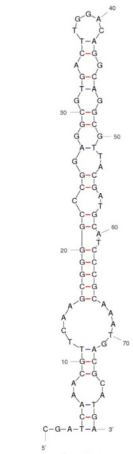
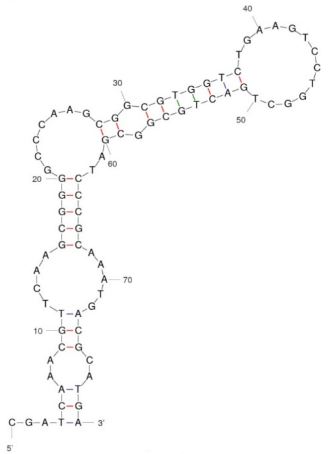
In development: Aptamer-based RT-qPCR Assay - testing aptamers to capture intact genotypes of HuNoV





APTL-1 △G=-11.04



ATPL-6 △G=-11.09

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Comparison: Pig Mucin vs Aptamer as receptor for Encapsulated HuNoV in the In Situ Capture PCR method

- Ct values: results vary with genotype

Table 2 Detection of clinical samples by APT-ISC-RT-qPCR comparing with PGM

Cq	3009	3010	4	17	3035	Negative control
PGM	28.38 ± 0.39^{c}	31.59 ± 0.78^{b}	33.87 ± 0.47^{b}	30.10 ± 0.01^{c}	33.42 ± 0.38^{c}	ND
APT M6-2	29.36 ± 0.26^{b}	32.59 ± 0.71^{ab}	32.31 ± 0.94^{bc}	30.81 ± 0.14^{b}	39.61 ± 0.30^{a}	ND
APTL-1	29.45 ± 0.23^{ab}	32.95 ± 0.16^{b}	32.84 ± 0.03^{c}	30.70 ± 0.25^{b}	33.68 ± 0.60^{bc}	ND
APTL-6	29.88 ± 0.21^{a}	33.49 ± 0.44^{a}	38.01 ± 0.86^{a}	32.54 ± 2.30^{a}	34.46 ± 1.16^{b}	ND

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Problem: Classic primer sets inefficient with various genotypes
Solution: Redesigned RT-qPCR primer/probe sets for the detection of GI and GII HuNoVs

Target	Name	Sequence(5'-3')	Polarity
GI	LZIF	TGTGGACAGGAGATCGCRATCT	+
	LZIR-A	CTCYGGTACCAGCTGGCC	-
	LZIR-B	CCTCYGGHACCAGCTGACC	-
	LZIP	HEX-CGTCCTTAGACGCCATCATCATTTAC-MGB	-
GII	LZIIF-A	GTGGGATGGACTTTTACGTGCCAAG	+
	LZIIF-B	GGTGGMATGGATTTTTACGTGCCCAG	+
	LZIIR	CGTCAYTCGACGCCATCTTCATTCAC	-
	LZIIP	FAM-AGCCAGATTGCGATCGCC-MGB	-

Redesigned vs. Kageyama RT-qPCR primers/probe(s) sets (Ct values: measuring HuNoV in clinical samples)

No.	Sample	Genotype	New Primer/Probe	Kageyama assay
1	57404	GI.1	18.45	30.11
2	14151	GI.2	32.36	NA
3	3010	GI.3	23.38	32.26
4	58407	GI.4	32.66	NA
5	57565	GI.8	27.25	NA
6	17151101	GII.2	20.98	NA
7	17152TXZ	GII.2	27.02	30.55
8	15651006	GII.3	22.69	25.28
9	16651029	GII.4	20.62	22.90
10	57395	GII.4	26.48	28.31
11	17151116	GII.4	20.79	23.07
12	58037	GII.4	29.38	31.90
13	1717	GII.4	19.30	21.54
14	1704	GII.4	24.12	24.13
15	3143	GII.4	25.69	28.02
16	1028	GII.4	28.91	30.43
17	3035	GII.4	26.35	27.24
18	3009	GII.4	19.09	23.42
19	17151030	GII.6	24.58	26.16
20	57417	GII.12	16.26	18.72
21	C7	GII.14	15.97	19.27
22	15651202	GII.17	29.17	31.18
23	3014	GII.PE	23.57	25.21
24	NEG		NA	NA

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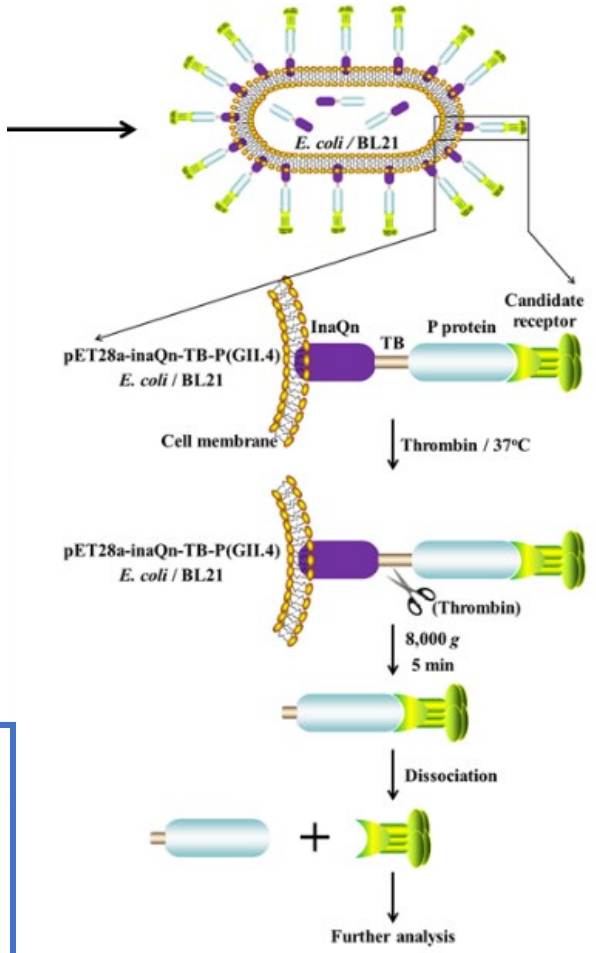
A Bacterial Surface Display System Expressing Cleavable Capsid Proteins of Human Norovirus: A Novel System to Discover Candidate Receptors in Multiple Systems (in progress)

- Recombinant *E. coli* BL21
- InaQn: Ice nucleation protein bound to a linker followed by HuNoV P protein
- HuNoV P protein binds to candidate receptors
- Cut at the linker
- Dissociate candidate receptor from P protein

- Using system to study candidate receptors on lettuce and on lettuce-associated bacteria



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Characterization of binding and release conditions between bacteria/recombinant-expressed HuNoV-capsid-P-protein complex (BPC) to Romaine lettuce extract

1. HBGA-like molecules present on Romaine lettuce needed for binding of BPCs, although other factors (pH, ionic strength) influenced binding efficiency.

2. Bound BPCs could not be released by free HBGAs in wash solution.

3. Bound BPCs were not removed by simply washing in water, but could be released by extremes of pH, high salt, and 0.1% (v/v) Tween-80.

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Further - Characterization of bacterial/viral interaction

- Isolation of HuNoV-binding bacteria from Lettuce
- Characterization of bacteria type, HBGA expression, HuNoV binding
- Characterization of molecules in bacteria for HuNoV binding

Characterization of Ecological Fitness of STEC – Diversity and adaptation within clonal groups in the produce production continuum

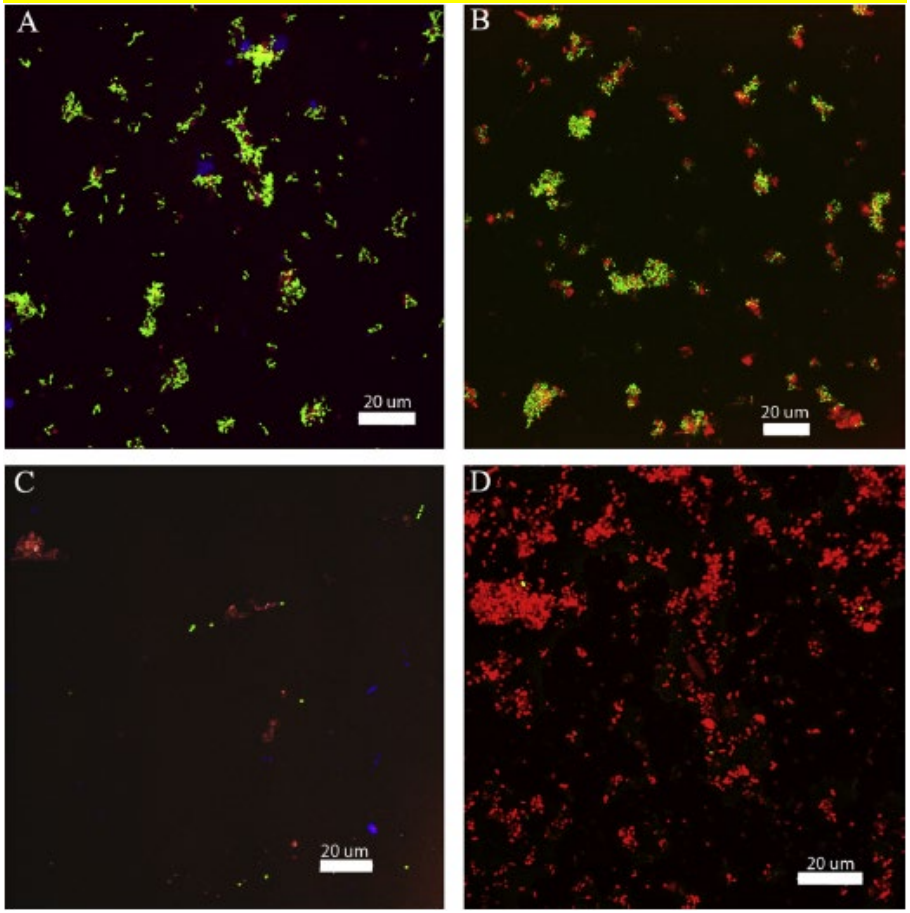
Michelle Qiu Carter
Produce Safety and Microbiology Research Unit, Albany, California

- There is heterogeneity within clonal groups of STEC
- Biological approaches to identify factors that contribute to survival and growth of STEC in produce
- Genomics approaches to identify genetic elements that shape the fitness of STEC in produce

Niche- and strain specific role of curli fimbriae in STEC

In collaboration with Maria Brandl (ARS, WRRC) and Indira Kudva (ARS, NADC)

Curli enhanced the mixed biofilm formation by STEC with spinach leaf associated microflora



O157:H7 outbreak strain Curli^{+}

Isogenic Curli^{-} mutant

Curli is an example of population heterogeneity – it's niche dependent

Mutations affecting curli production (transcriptional regulators) are widespread in the wild STEC population.

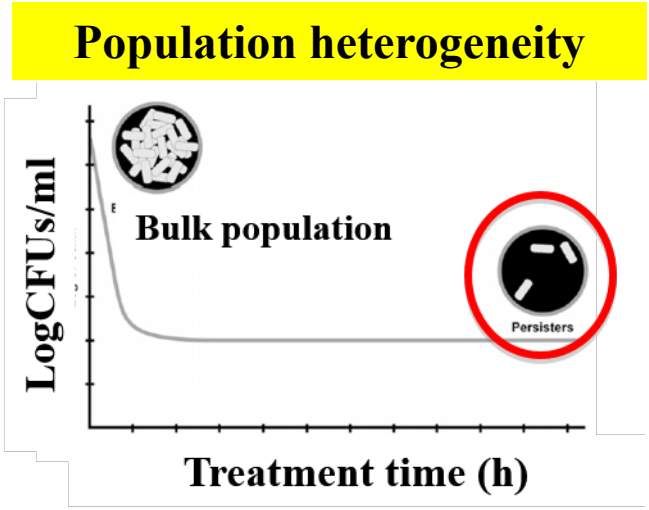
What role in produce association?

Curli necessary for biofilm formation on glass with spinach leaf flora

Carter et al 2016 Food Micro

Role of persisters in STEC environmental survival

In collaboration with Maria Brandl (ARS, WRRC)



Kint et al 2012 Trends Microbiol

Persister cells – Another example of natural heterogeneity within clonal populations of STEC

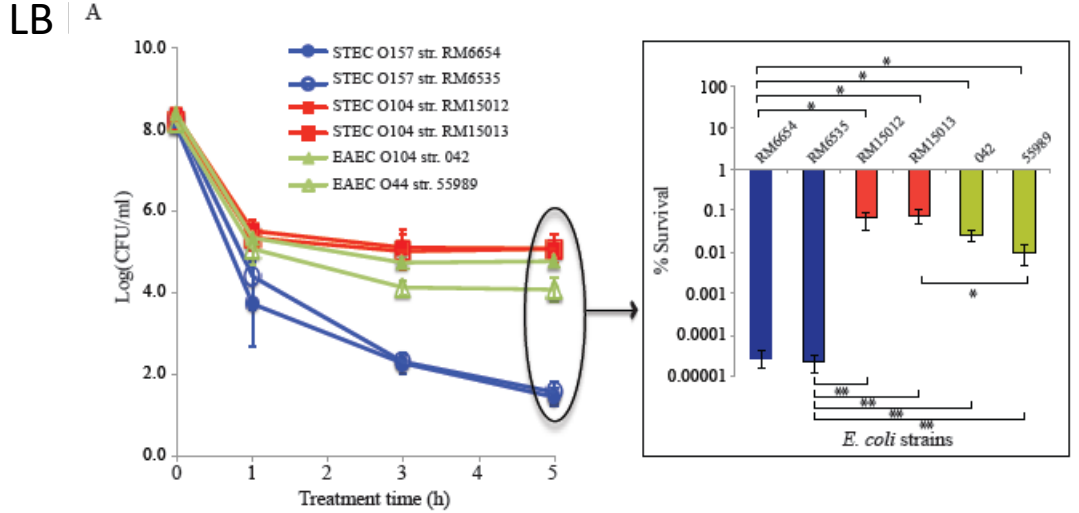
They form naturally within STEC populations – they have high tolerance to antibiotics and other stresses.

Can enumerate the persister population in very late stationary phase by lysing metabolically active cells with high doses of ciprofloxacin

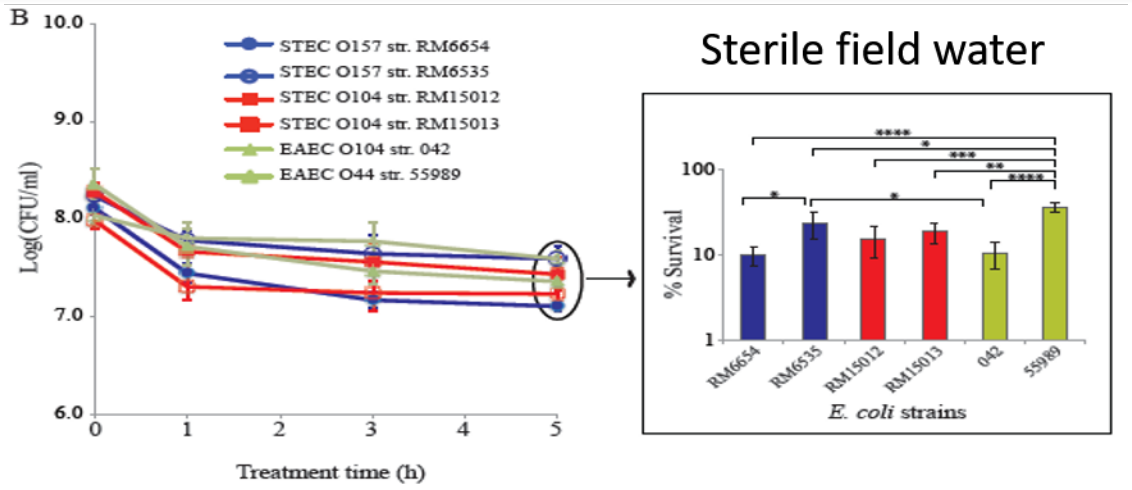
Persister cell formation in environmental and clinical STECs

Strain differences in persister formation

More persisters formed in field water



- The persister fraction of STEC in exponential-phase of culture varied greatly among the strains examined (0.00003% - 0.08%)



- The highest persister fraction was detected in populations of STEC cells incubated in field water (9.9-23.2%)

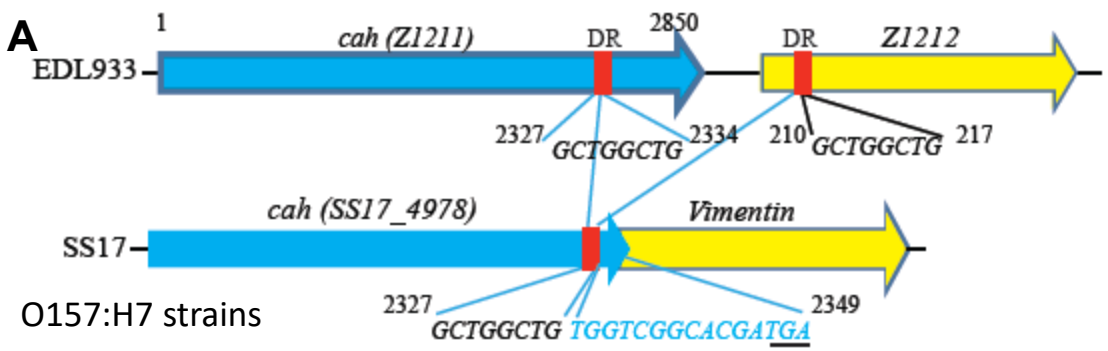
Current focus

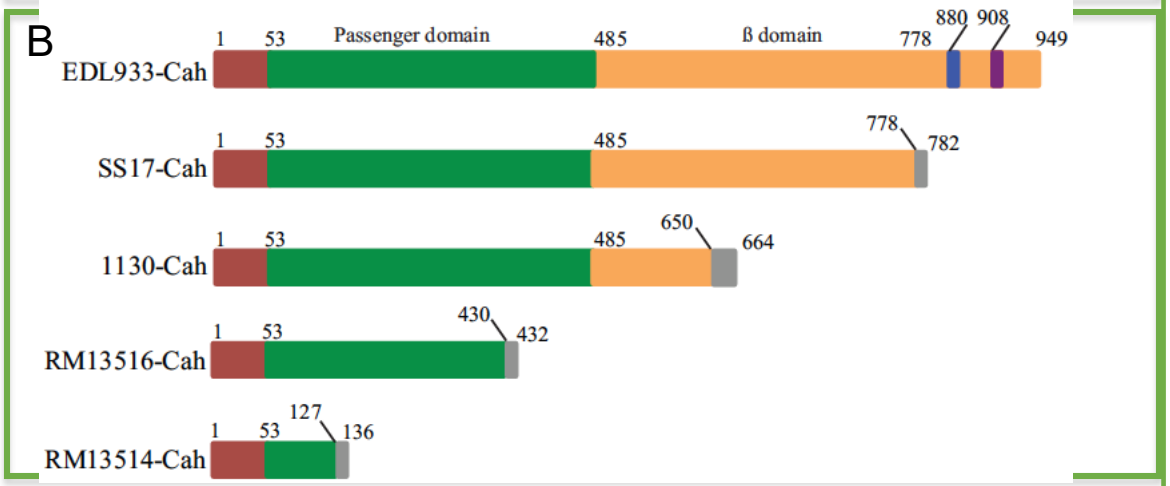
1. Investigate the STEC persister populations on leafy greens under different growth conditions.

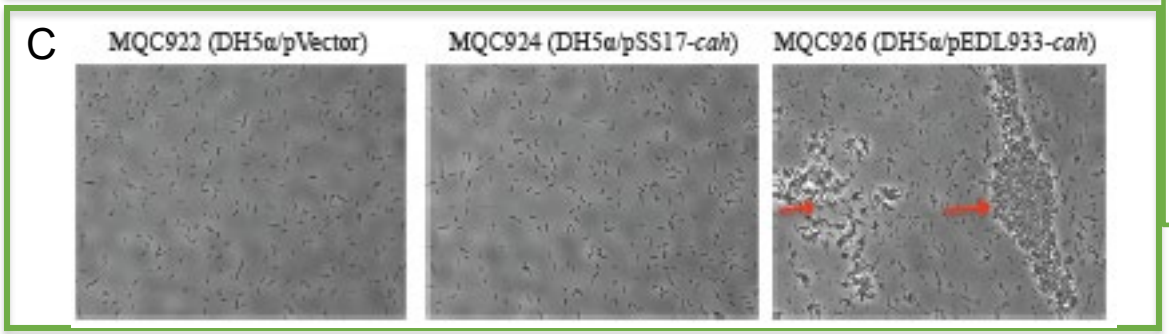
2. Develop mathematical models to predict the STEC persister populations in field growth conditions.

Variants improve STEC ecological fitness – *cah* gene

In collaboration with Maria Brandl (ARS, WRRC); Indira Kudva (ARS, NADC); and Vivek Kapur, PSU







- *Cah* mutation is common in STECs (~32%)
- *Cah* (surface protein, similar to Ca-binding prot) first identified in EDL933
- *Cah* deleted in SS17 (supershedder), present in EDL933
- *Δcah* in strain EDL933 → enhanced attachment to spinach leaves
- *Cah* → SS17 strain →increased attachment to leaf surfaces
- Strain differences in role of *cah.* Ecological function of *cah* modulated by environmental conditions and other bacterial properties.
- *Cah* and Curli show adaptive mutation is means to ensure survival of STECs.

Carter et al 2017 AEM

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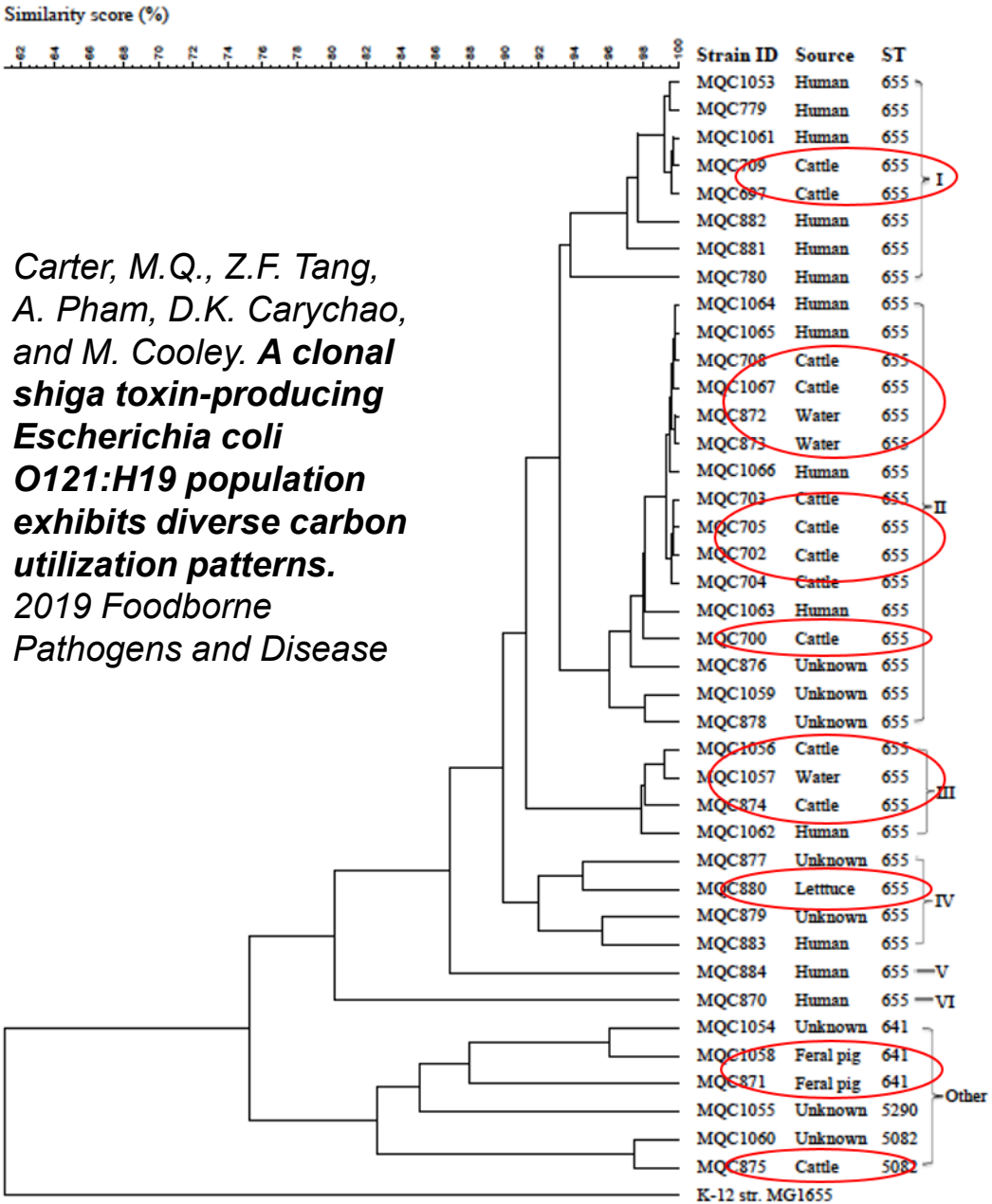
Divergent carbon utilization in a STEC clonal population: O121



Carter, M.Q., Z.F. Tang, A. Pham, D.K. Carychao, and M. Cooley. ***A clonal shiga toxin-producing Escherichia coli O121:H19 population exhibits diverse carbon utilization patterns.*** *2019 Foodborne Pathogens and Disease*

Omnilog: 190 different Carbon sources
All strains of O121:H19

Key points

1.The STEC O121 strains with the same genotype were clustered together based on the carbon utilization pattern.

2.Carbon substrates exhibiting lineage (ST) and strain specific metabolic profiles were identified.

3.Although clinical ST-655 strains displayed higher metabolic activity than environmental ST-655 strains for several carbon substrates, a few environmental strains with the enhanced metabolic potential for these substrates were also detected.

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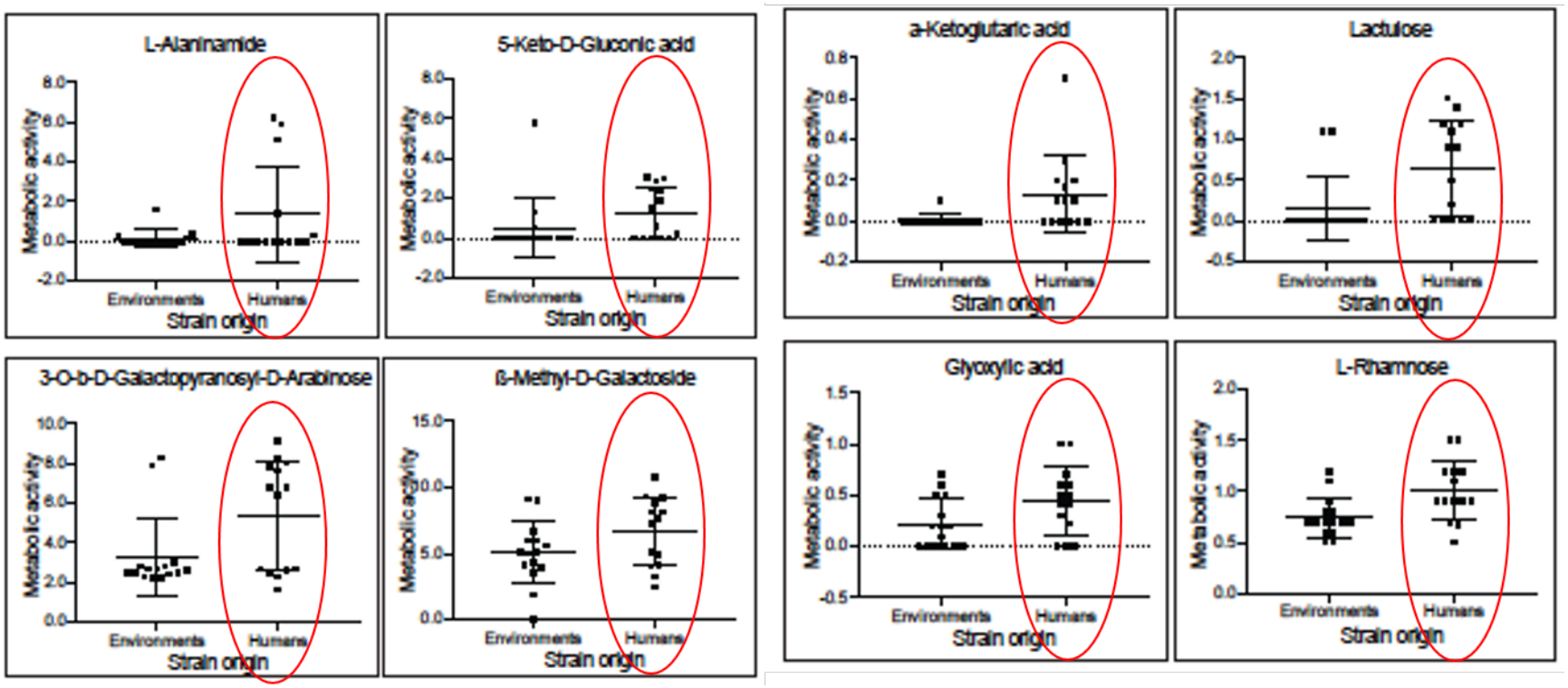
Enhanced metabolic activity in 14 clinical strains vs 15 environmental strains in C substrates– Niche adaptation?



14 clinical strains & 15 environmental ST-655 strains (O121)

Carter, M.Q., et al. 2019 Foodborne Pathogens and Disease

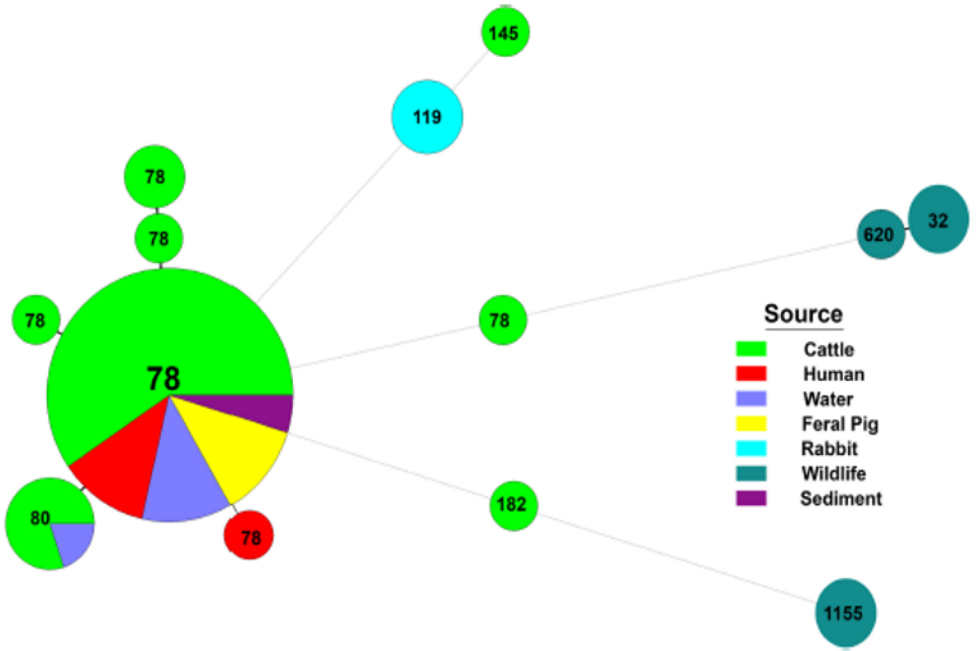
Current focus

In collaboration with Craig Parker at ARS, WRRC, we are identifying genes/pathways that contribute to 1) lineage and niche specific carbon utilization pattern; 2) differential utilization of above carbons substrates between clinical and environmental strains.

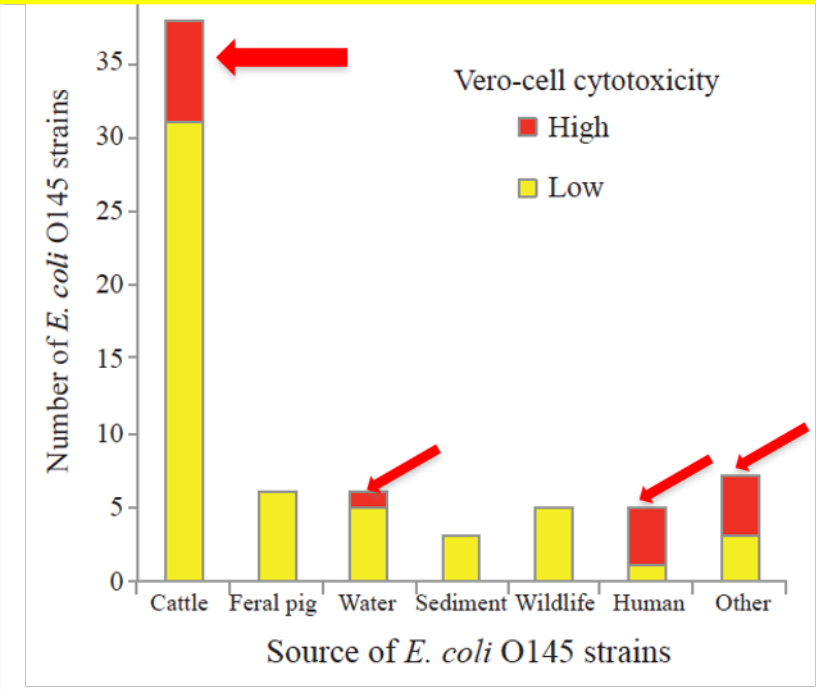
Divergent evolution of STEC in STEC clonal populations: O145 shiga toxin cytotoxicity

In collaboration with Beatriz Quinones, Xiaohua He, Robert Mandrell, and Michael Cooely (ARS, WRRC)

A. Environmental STEC O145 strains display clonal structure via 7 gene MLST



B. Cytotoxicity of shiga toxins in environmental O145 strains varies



Although all core virulence gene are conserved in majority of strains, only a subset of cattle isolates exhibited key virulence traits comparable to those of the STEC O145 outbreak strains

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Differential induction of shiga toxin-converting prophages in environmental STEC strains (WGS of 12 environmental strains)

ID	Strain	Source	Serotype	ST	Cytotoxicity	Genome		
						Chromosome	#Plasmids	#Stx-prophages
MQC634	RM8843	Cattle, CA	O145:H28	78	L	5,458,415	1	1
MQC642	RM8988	Cattle, CA	O145:H28	78	L	5,458,186	1	1
MQC644	RM8995	Sediment, CA	O145:H28	78	L	5,457,980	1	1
MQC662	RM11626	Cattle, CA	O145:H28	78	L	5,419,044	1	1
MQ774	RM12275	Sediment, CA	O145:H28	78	L	5,408,598	1	1
MQC646	RM9154	Cattle, CA	O145:H28	78	H	5,205,721	3	1
MQC650	RM9467	Cattle, CA	O145:H28	80	H	5,385,895	1	1
MQC651	RM9872	Cattle, CA	O145:H28	80	H	5,385,904	1	1
MQC652	RM9873	Cattle, CA	O145:H28	80	H	5,385,819	1	1
MQC665	RM12367	Cattle, CA	O145:H28	80	H	5,472,396	1	2
MQC729	RM10425	Cattle, CA	O145:H28	80	H	5,343,037	1	1
MQC80	RM13514	Human	O145:H28	78	H	5,585,613	2	1
MQC77	RM13516	Human	O145:H28	78	H	5,402,276	2	1

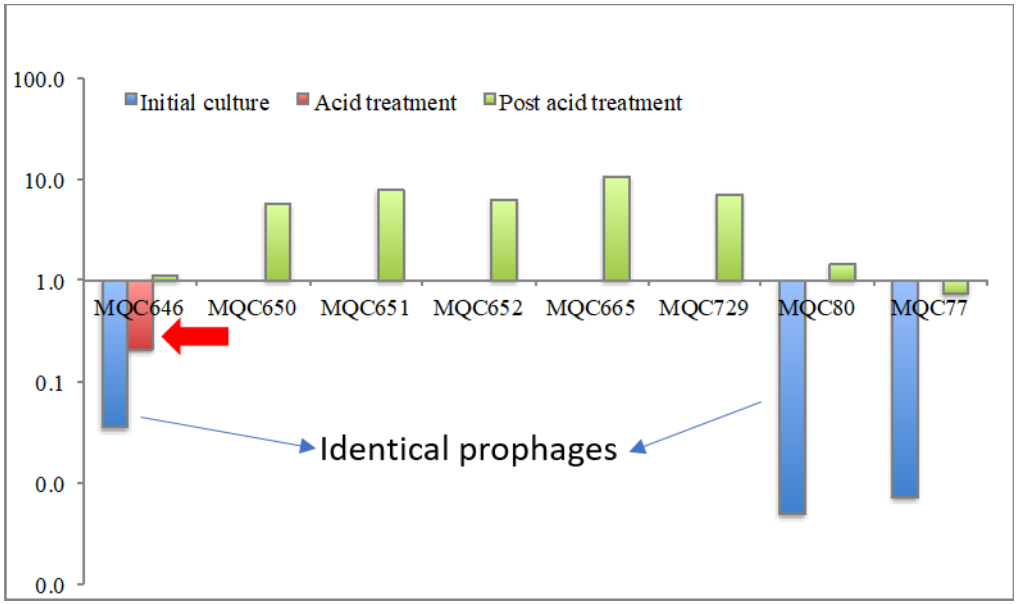
Induction of shiga toxin-converting prophages in environmental STEC strains

In collaboration with Xiaohua He, (ARS, WRRC)

Key points

- All environmental strains exhibiting high cytotoxicity carry one or multiple Stx2a-converting prophages.
- Induction of Stx2a by MMC and acid challenge (pH 2.5 at 37°C for 2 h) in environmental strains is comparable to the two outbreak strains (MQC80 and MQC77)
- Strain variation in Stx production was observed prior to and following MMC and acid challenge.

C. Stx2a production following acid challenge



Genetic diversity of STEC in other environmental reservoirs

In collaboration with Michael Cooley (ARS, WRRC)

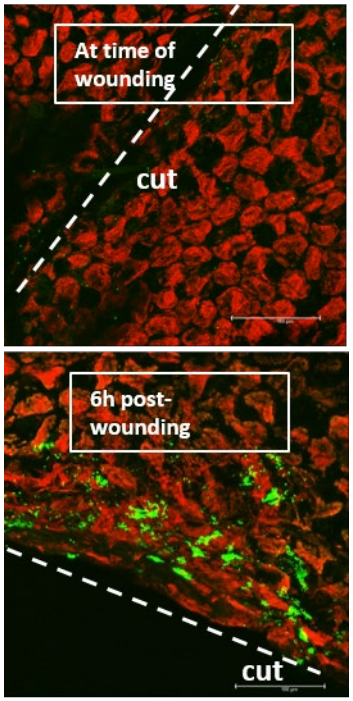
Strain ID	Phylogroup	stx1(cfr)	stx2(f)	stx2(abc)	stx2(ex)
1	A	16.6	No Ct	No Ct	No Ct
2	Clade III, IV, or V	No Ct	16.57	No Ct	No Ct
3	C	No Ct	No Ct	No Ct	No Ct
4	Clade III, IV, or V	No Ct	17.03	No Ct	No Ct
5	Clade III, IV, or V	No Ct	16.91	No Ct	No Ct
6	Clade III, IV, or V	No Ct	16.89	No Ct	No Ct
7	C	No Ct	No Ct	13.97	No Ct
8	Clade III, IV, or V	No Ct	18.21	No Ct	No Ct
9	Clade III, IV, or V	No Ct	17.26	No Ct	No Ct
10	Clade III, IV, or V	No Ct	18.05	No Ct	No Ct
11	Clade III, IV, or V	No Ct	17.55	No Ct	No Ct
12	Clade III, IV, or V	No Ct	15.72	No Ct	No Ct
13	D	No Ct	No Ct	17.79	18.71
14	A	No Ct	No Ct	20.42	20.12
15	Clade III, IV, or V	No Ct	16.93	No Ct	No Ct
16	Clade III, IV, or V	No Ct	17.25	No Ct	No Ct
17	Clade III, IV, or V	No Ct	17.79	No Ct	No Ct
18	Clade III, IV, or V	No Ct	17.64	No Ct	No Ct
19	Clade III, IV, or V	No Ct	17.57	No Ct	No Ct
20	Clade III, IV, or V	No Ct	17.64	No Ct	No Ct

Genomic information	RM9973 (ID=4)	RM10410 (ID=7)
Serotype	O55:H52	O113:H4
Phylogroup	Clade III, IV or V	C
stx genotypes	$stx2_f$	$Stx1_a$ $stx2_{d2}$
Chromosome	4,648,335 bp	5,227,472 bp
Plasmid1	105,082 bp	NA
Plasmid2	102,351 bp	NA
#Prophages	5	7
Stx2f-prophage	45,000 bp	NA
Stx1a-prophage	NA	62,330 bp
Stx2d2-prophage	NA	58,193 bp

Current focus

- STEC isolated from birds appear to belonging to different lineages compared with those from cattle or other environmental sources.
- Do birds serve as a natural reservoir of hyper-virulent STEC strains?

EcO157 imports plant-synthesized choline for use in adaptation to osmotic stress

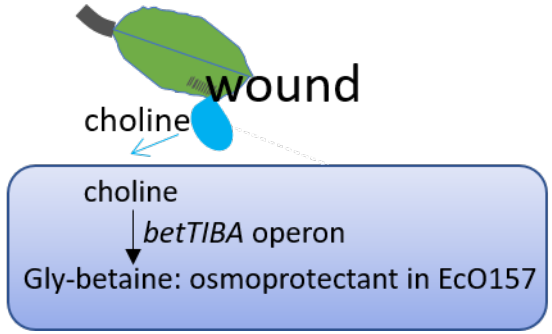


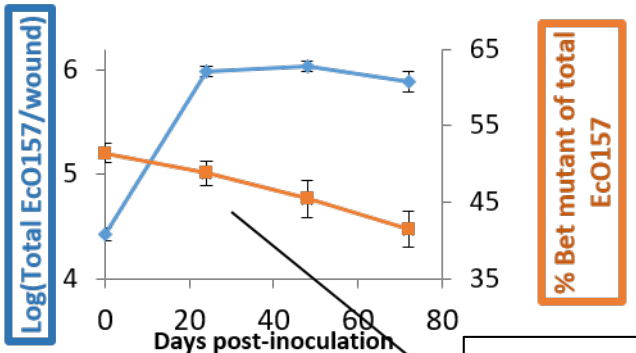
Wounded lettuce leaf

Transcriptome in 2 lettuce systems (romaine lettuce lysate, MAP iceberg shredded)

1) Upregulation of osmotic stress response operon *betTIBA* for production of glycine-betaine as an osmoprotectant

EcO157 uses plant-derived choline for osmoprotection in lettuce leaf wounds





ΔbetTIBA is less fit in lettuce wounds

Decreasing competitive fitness of mutant vs WT in lettuce wounds

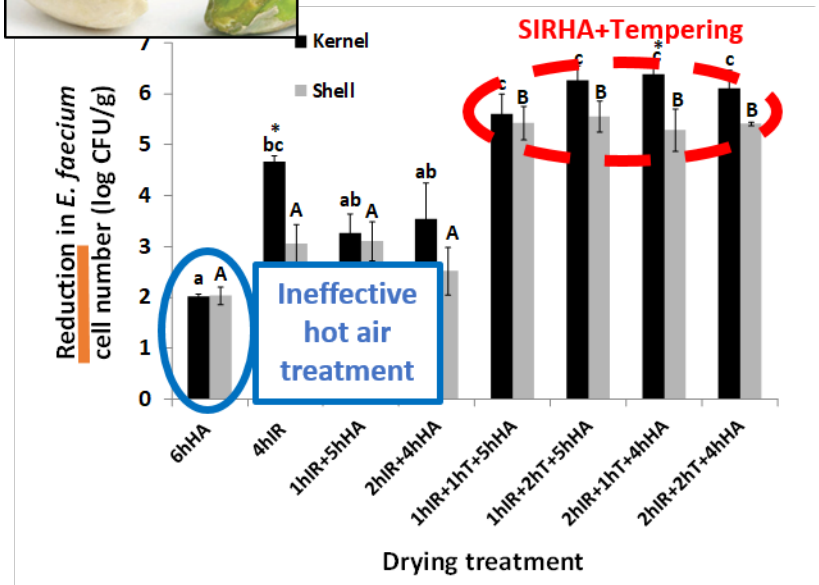
Impact: Despite potential for multiplication in cut lettuce, EcO157 experiences a variety of stresses, some of which it adapts to by exploiting the plant habitat itself. **Would differences in lettuce physiology and composition affect this tolerance of EcO157 to stress in processed lettuce, and could this be mined to improve produce safety?**

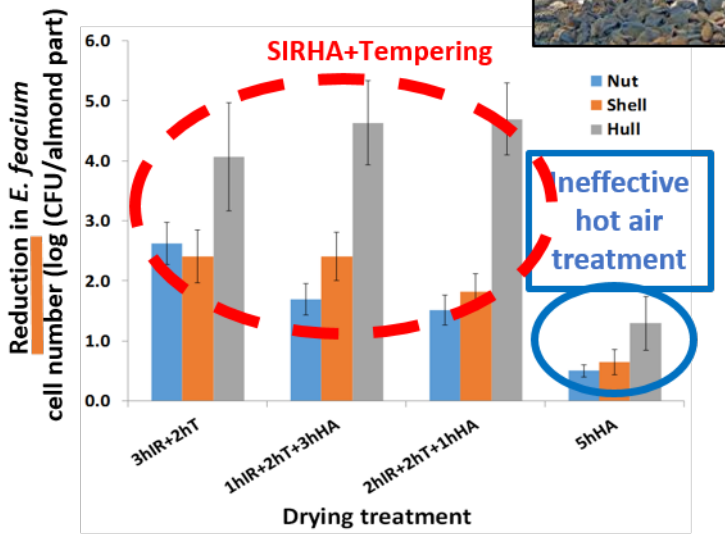
Brandl lab

Efficacious and Energy-saving Technology to Dry and Sanitize In-shell Pistachios and Whole Almonds

(in collaboration with Zhongli Pan, Healthy Processed Foods Research Unit, ARS, WRRC, CA)

Problem: 1) Pistachios sorted for quality in water tanks and must be dried before further processing
2) Almonds get wet during rain when piled up in the orchard and must be dried before processing
3) Both nuts have been associated with salmonellosis outbreaks/recalls





Impact: Faster drying (e-saving) and greater sanitization efficacy of wet pistachio and almonds by combining IR heat, tempering and hot air than by traditional hot air alone

Persistence of pathogens in the environment

Mike Cooley

Development of a droplet digital PCR method to monitor the levels of STEC, *Listeria monocytogenes* and *Salmonella* in environmental samples.

1. Quantification of pathogen levels in environmental samples is costly and labor intensive.
2. Originally developed to augment incident data on these pathogens from a 5 year FDA study of watersheds in the Salinas region.
3. Published method will support further research on transport and persistence

Optimizing DNA co-extraction of pathogens from surface water samples – Percent of spiked cells detected by ddPCR using different DNA extraction methods

	Spiked cell detection of virulence genes (%)			
Method	*stx*	*ehx*	*invA*	*hly*
MoBio Basic	15	40	13	1.5
+ Lysozyme	49	86	26	1.1
+ Enzyme mix	27	42	24	6
+ Beadbeat (BB)	83	133	28	5
+ Enzyme + BB	118	160	45	8
+ Sonication	92	83	58	101

Samples may contain multiple pathogens. To enumerate, you need to extract DNA from all types efficiently. Testing methods here with spiked water samples (10^4 CFU/ml). Detection of virulence genes by ddPCR

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Droplet digital PCR quantification of pathogens in Salinas surface water samples

36 water samples processed by culture methods and ddPCR

Organism	Target gene	PCR positive	Culture positive	Dice similarity coefficient[1]	Average template number (range)
Shiga toxin producing *E. coli* (STEC)	shiga toxin 2 (*stx2*)	21/36	19/36	85%	923 (0-20k)
	hemolysin (*ehx*)	29/36	19/36	75%	23k (0-753k)
Listeria	listeriolysin (*hly*)	21/36	22/36	79.1%	69 (0-362)
Salmonella	invasion protein A (*invA*)	30/36	27/36	87.7%	152 (0-517)

[1] Correlation between PCR positive and culture positive samples.

Cooley MB, Carychao D, Gorski L (2018) Optimized co-extraction and quantification of DNA from enteric pathogens in surface water samples near produce fields in California. Frontiers in Microbiology 9:448

Typing via WGS (MLST) of the STEC collection

As a result of several survey initiatives over the last 10 years we have a large collection of STECs isolated from the environment around the Central California Coast

- Typing via WGS (MLST) of the STEC collection to improve our understanding of persistence and transport of pathogens in the environment.
  - wgMLST has supplanted and refined MLVA typing
  - Many clusters showed hints of interesting persistence or transport.

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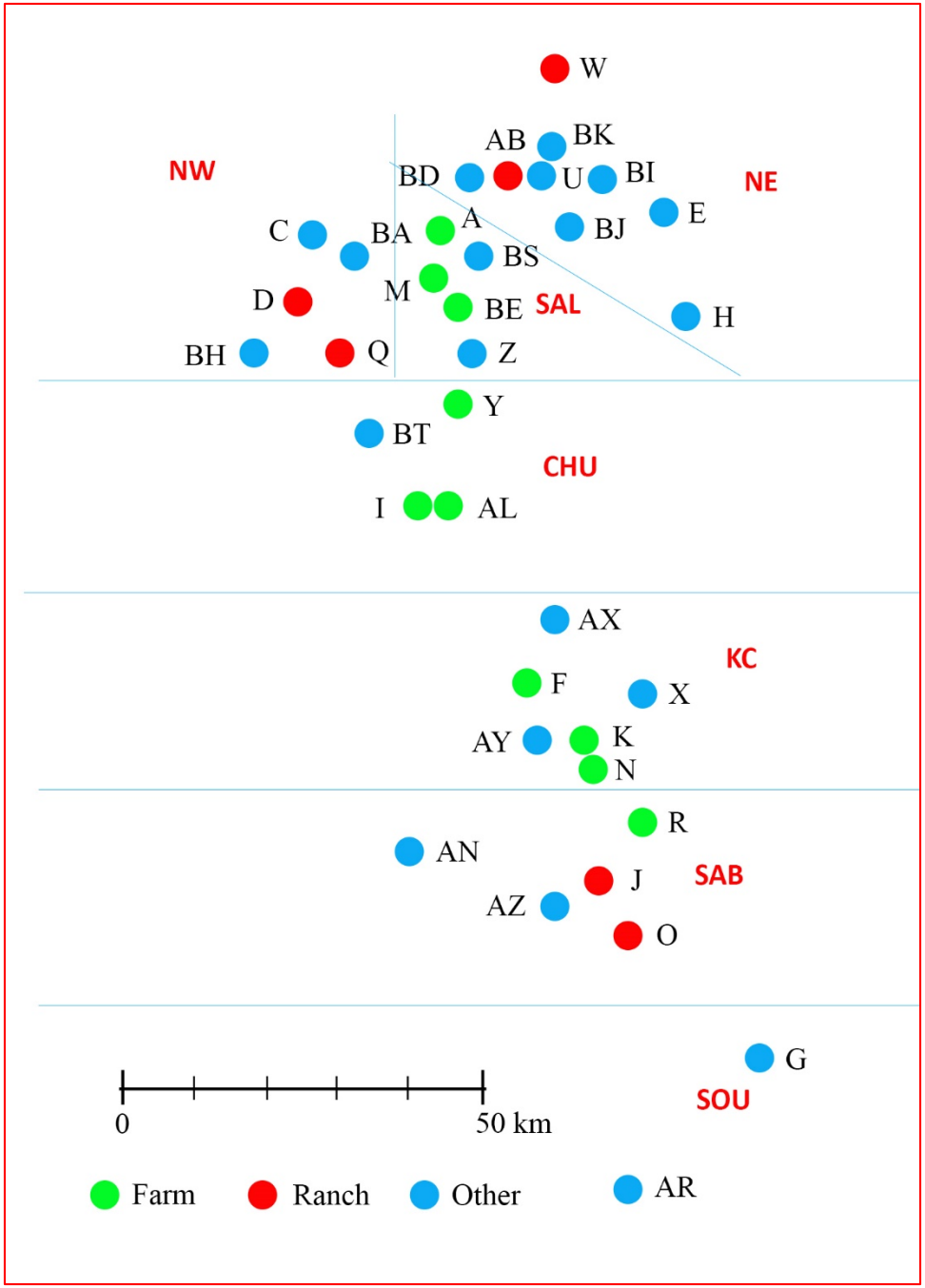
Map of sample sites divided by sample region and sample type

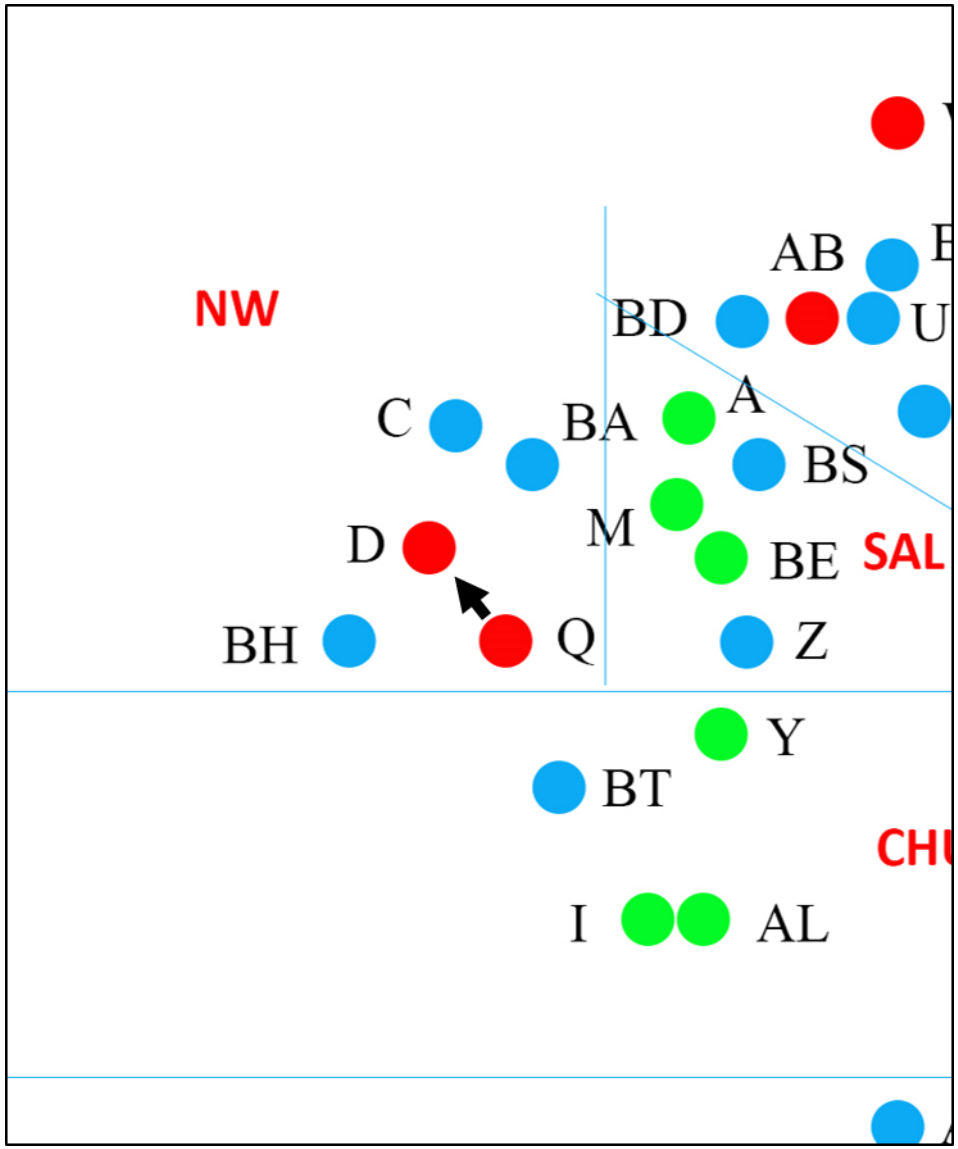
Actual sample locations unknown
Distances extracted from matrix



Example 1 of movement of STEC subtype from D → Q through the region over time

Cluster 66

- Initial samples at Ranch D and Ranch Q – negative
- Then: Feral pig at Q – positive for O157 and STEC
- Later: Many cattle positive at Q (2 months) and later at D (8 months) – movement of strain



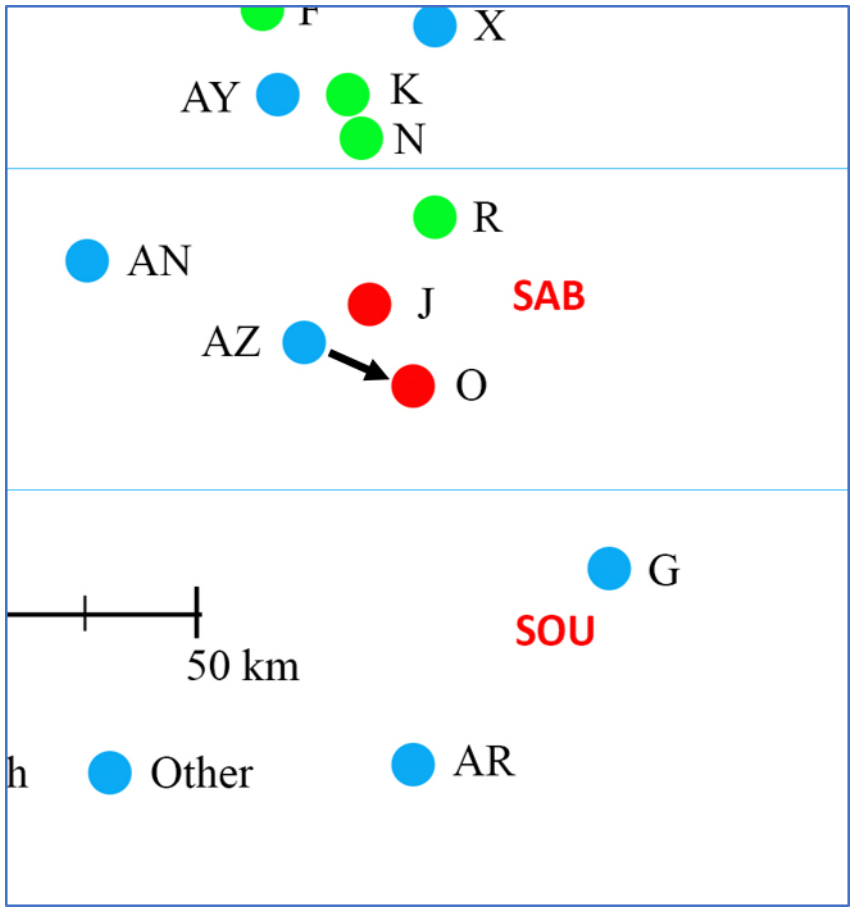
Example 2 of movement of STEC subtype from AZ → O through the region over time

Cluster 114

Found twice in sediment at AZ (two month period)

Later found in cattle at O (6 and 10 months later)

Other clusters being investigated – some more complicated involving widely spaced sampling sites and multiple years.

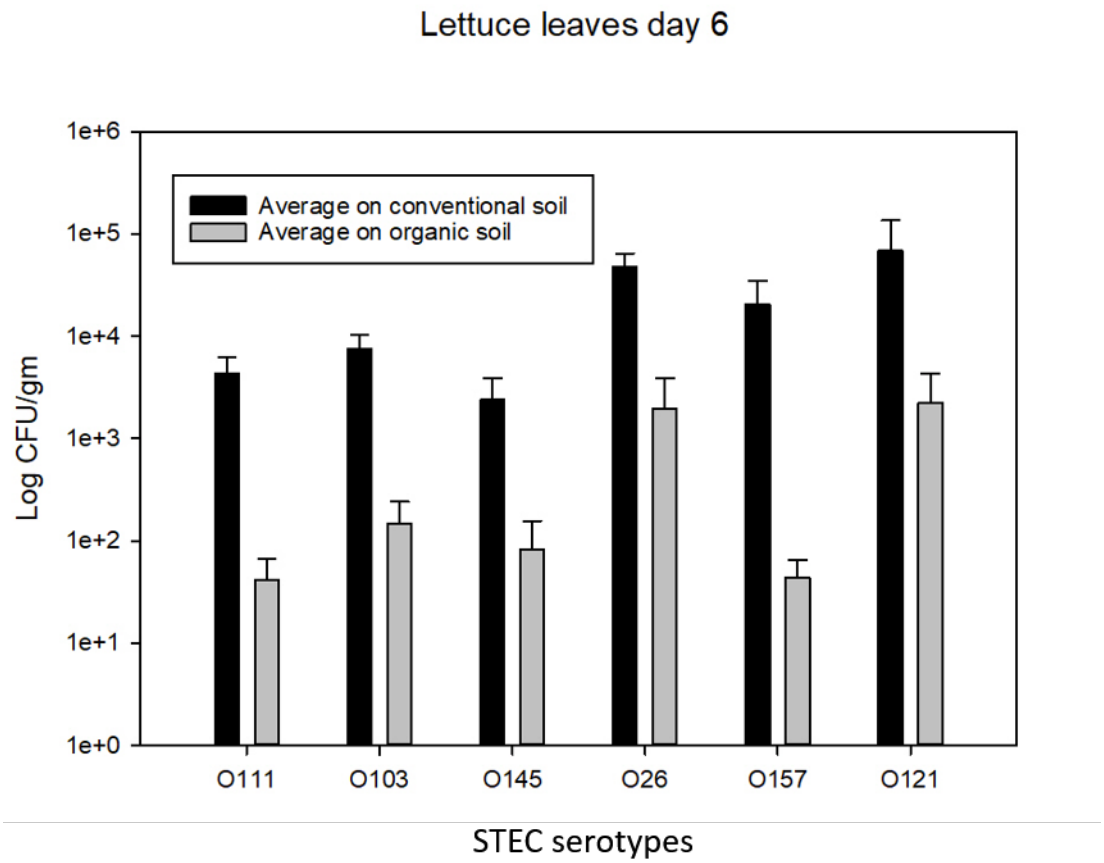


The effect of organically maintained soil on the persistence of human pathogenic bacteria in lettuce phyllosphere.

- Now in its third year in collaboration with UC Davis.
- Using soils strictly maintained as either organic or conventional for the past 12 years.
- Soils are removed from selected fields at various times and experiments are performed in growth chambers.

STEC competition differences in conventional and organic soils

Contaminated seed planted

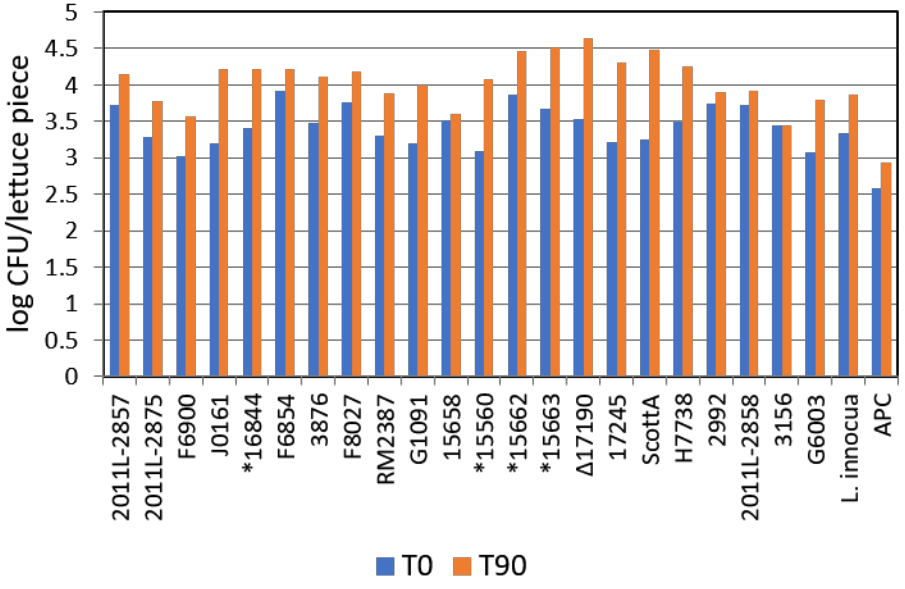


- All serovars showed less persistence on plants grown in organic soils.
- Presumably due to greater microbial activity in organic soils
- This result repeated with soils collected at different points during growing season.

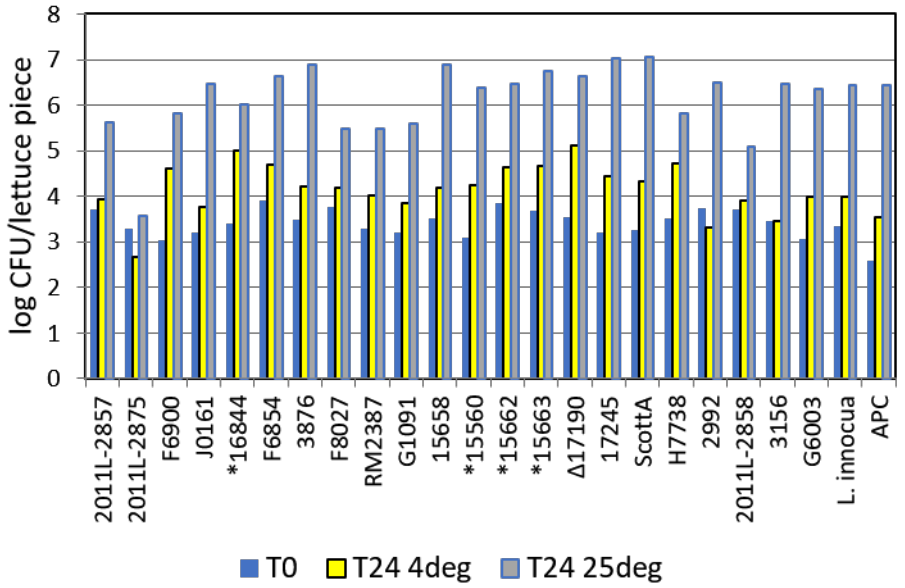
L. monocytogenes attachment and growth on produce and abiotic surfaces

In collaboration with Sophia Kathariou (North Carolina State)

Letttuce Attachment from 0 to 90 minutes

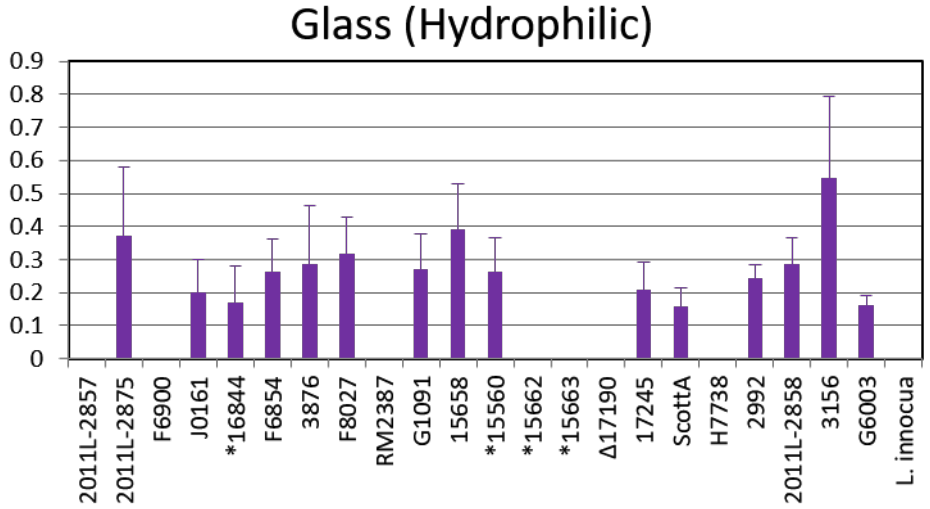


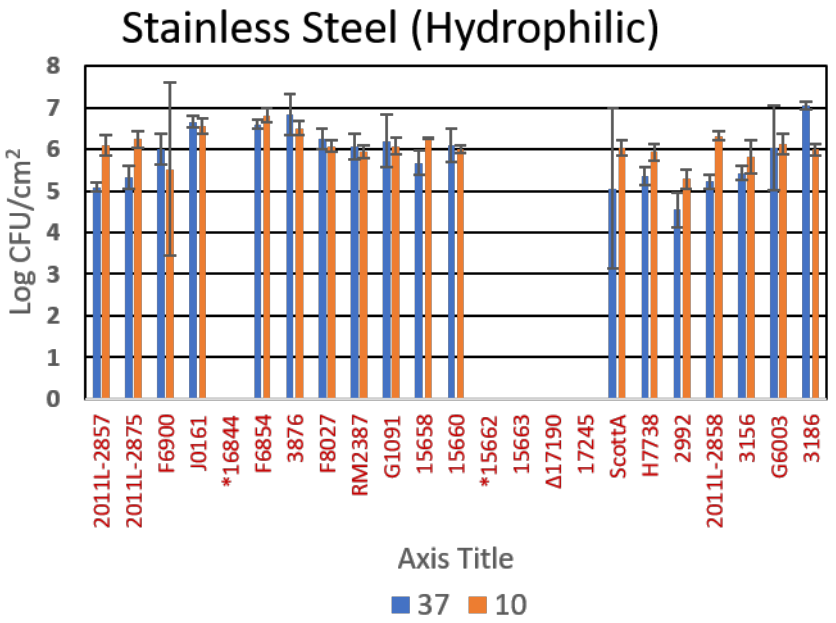
Lettuce Growth after attachment, 24h at 4 and 25 degrees

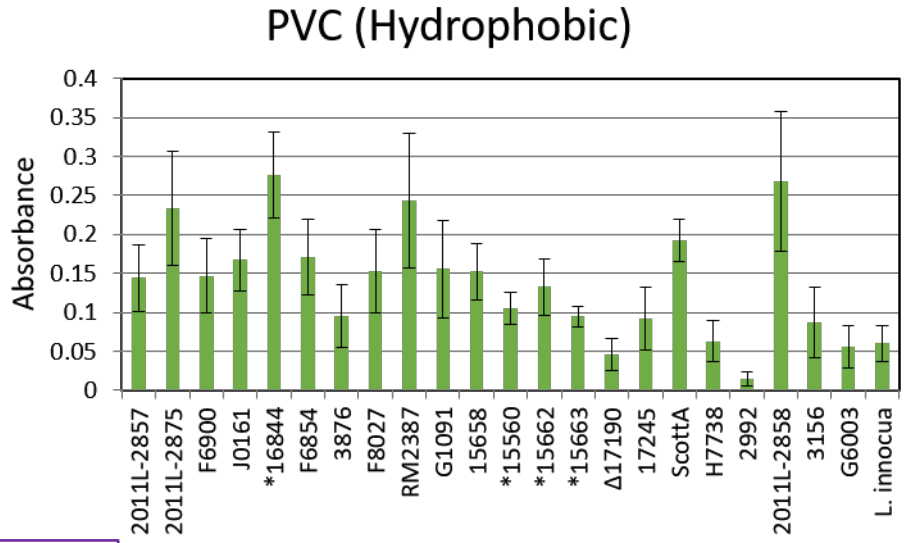


Strain differences in attachment and strain differences in growth.
Similar results with cantaloupe as an attachment/growth matrix

Surface attachment/growth of *L. monocytogenes* strains vary depending on surface type







- Strain differences in attachment and growth on different surface types
- What genes control the attachment and growth on the different surfaces?

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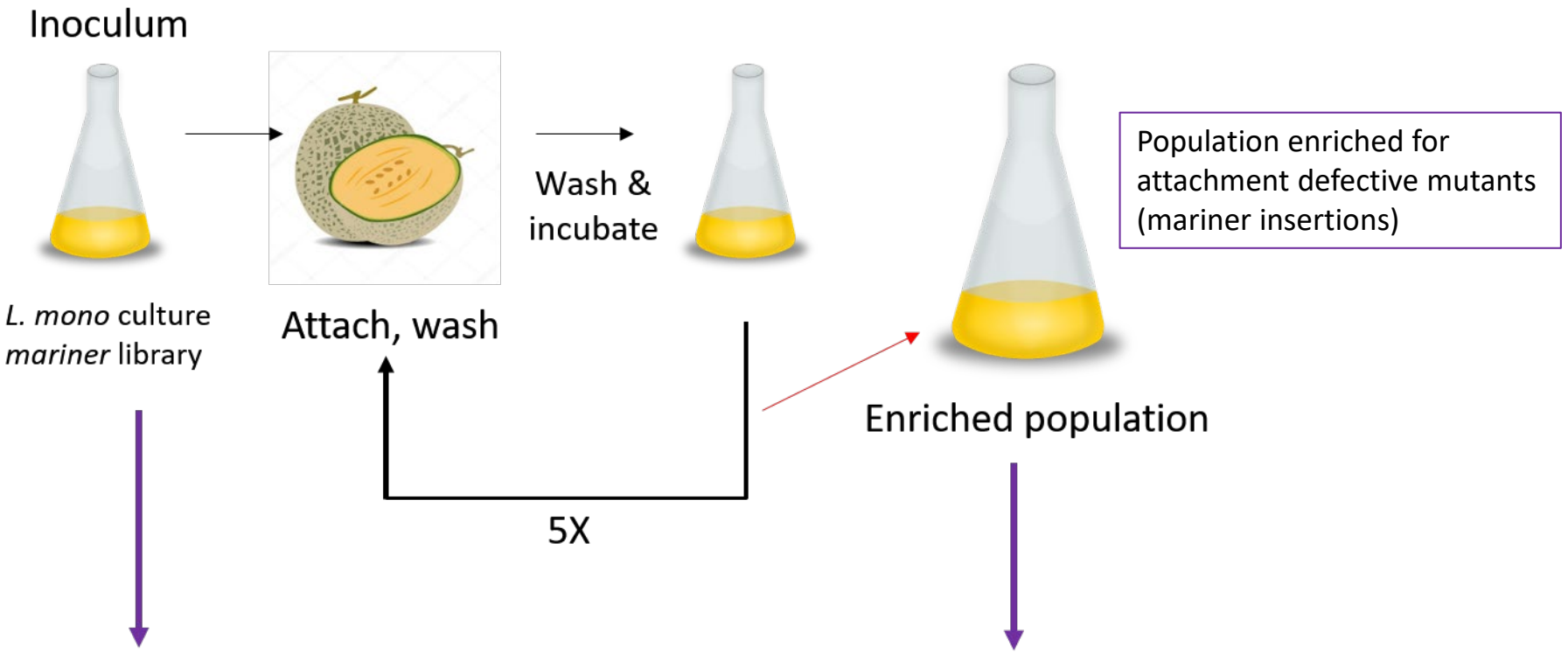
Amplicon sequencing to determine *L. monocytogenes* attachment and growth-related genes

In collaboration with S. Kathariou (North Carolina State) and W. Miller (ARS, WRRC)

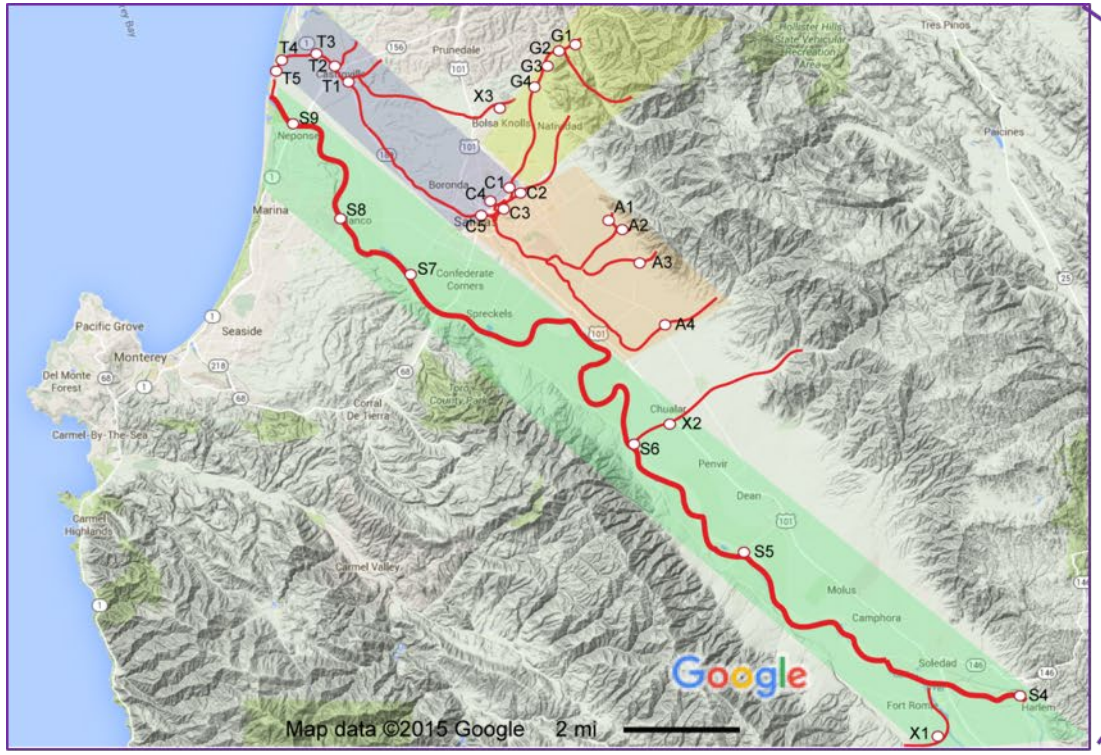


1) Make DNA, Sequence from transposon, Compare numbers of genes between pools

2) Genes needed for attachment will be present in a higher number in the enriched DNA pool

Pathogens in the Environment: Persistence and Ecology

Sampling Region around Salinas, California. Moore swab samples collected every 2 weeks over 5 years - > 3000 samples



500 sq. miles (1300 sq. km)

October 2011 – September 2016

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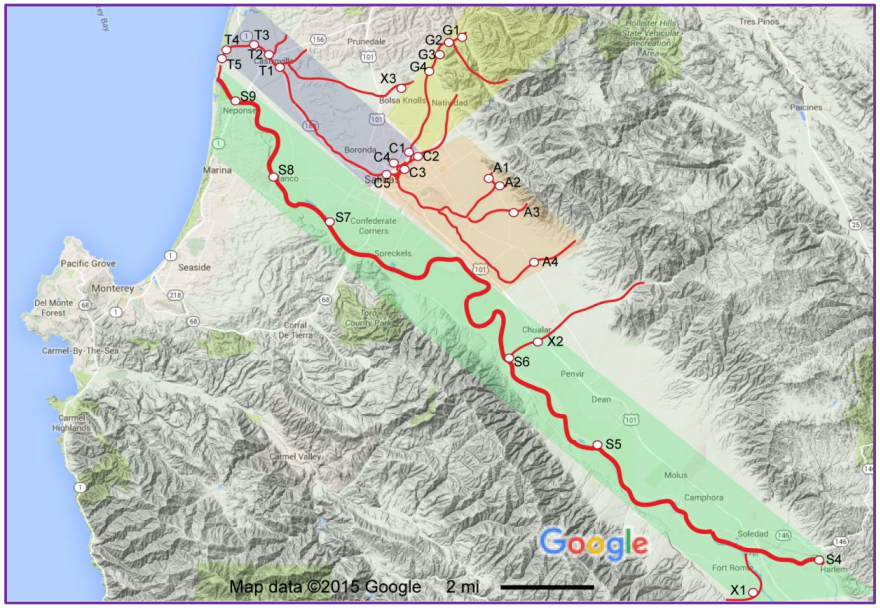
Prevalence and Location of *L. monocytogenes* and *Salmonella*

In collaboration with M. Cooley, USDA, ARS



Salmonella: Found everywhere, no statistical differences among locations.

L. monocytogenes: Found everywhere, more prevalent in Gabilan, Alisal, Tembladero, and Carr Lake

Prevalence by year

Year	*Salmonella*	*L. monocytogenes*
2011 (Oct – Dec)	45	21
2012	77	46
2013	77	42
2014	63	39
2015	45	43
2016 (Jan – Sep)	72	45
OVERALL	67	42

Prevalence by season

Season	*Salmonella*	L. *monocytogenes*
Fall	49 ± 13 A	29 ± 17 A
Winter	53 ± 20 AB	55 ± 20 B
Spring	64 ± 18 B	50 ± 17 B
Summer	54 ± 22 AB	29 ± 13 A

Cooley et al (2014) Front Cell Inf Microbiol 4:30

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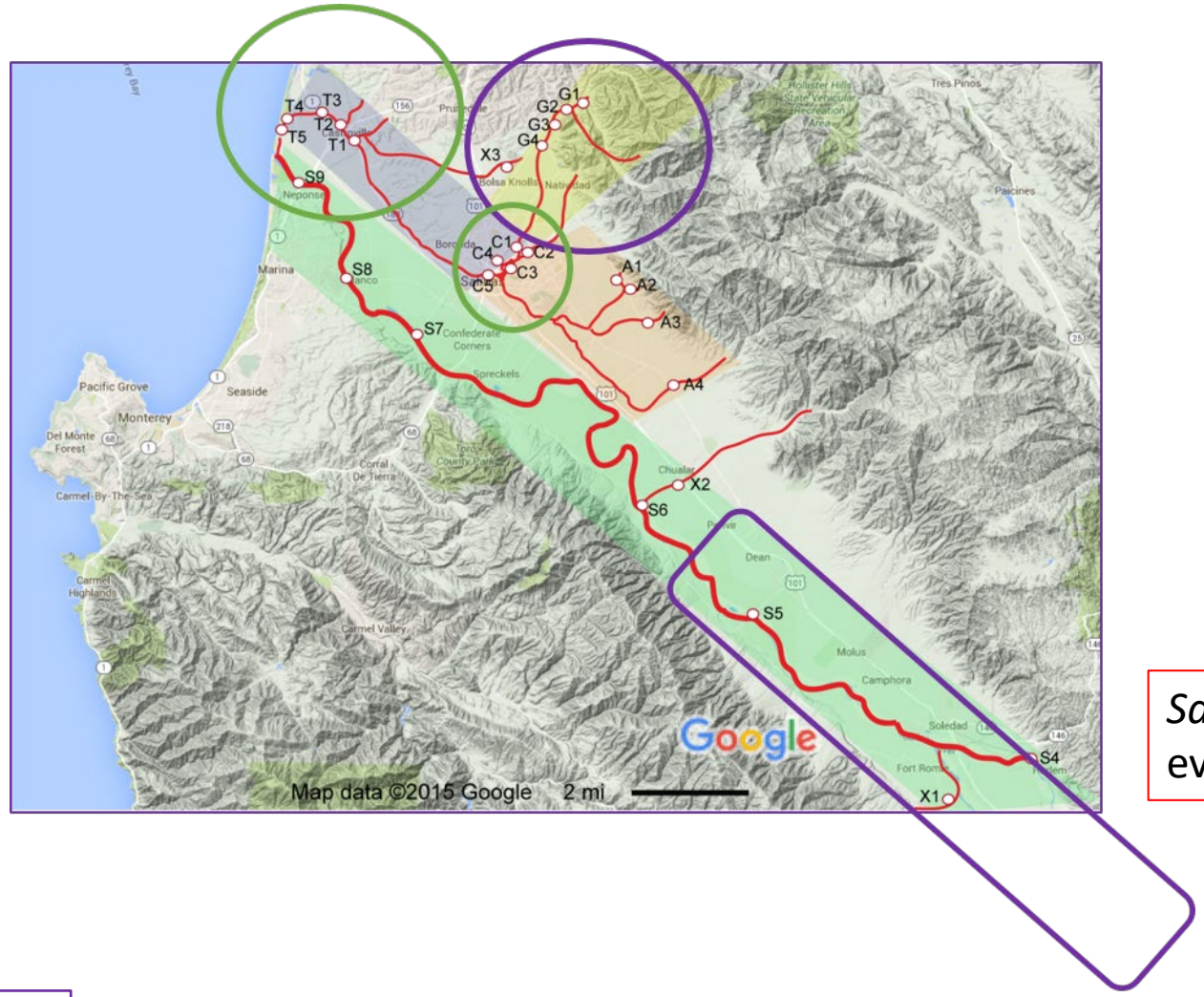
Top 20 serovars among *Salmonella* isolates (2,029 isolates, in process)

Serovar	Number of samples containing
6,8:d:- and Muenchen	485
Give	290
Typhimurium	203
Oranienburg	94
Montevideo	77
Infantis	38
Anatum	35
Heidelberg	32
Agona	27
Enteritidis	27
Thompson	27
Senftenberg	23
Newport	21
4,[5],12:i:-	19
Braenderup	19
Litchfield	19
Saintpaul	19
Berta	16
Reading	16
III_41:z4,z23:-	16

Some *Salmonella* serovars have location preferences



6,8:d:- and Muenchen

Typhimurium and Infantis

Salmonella Give is everywhere

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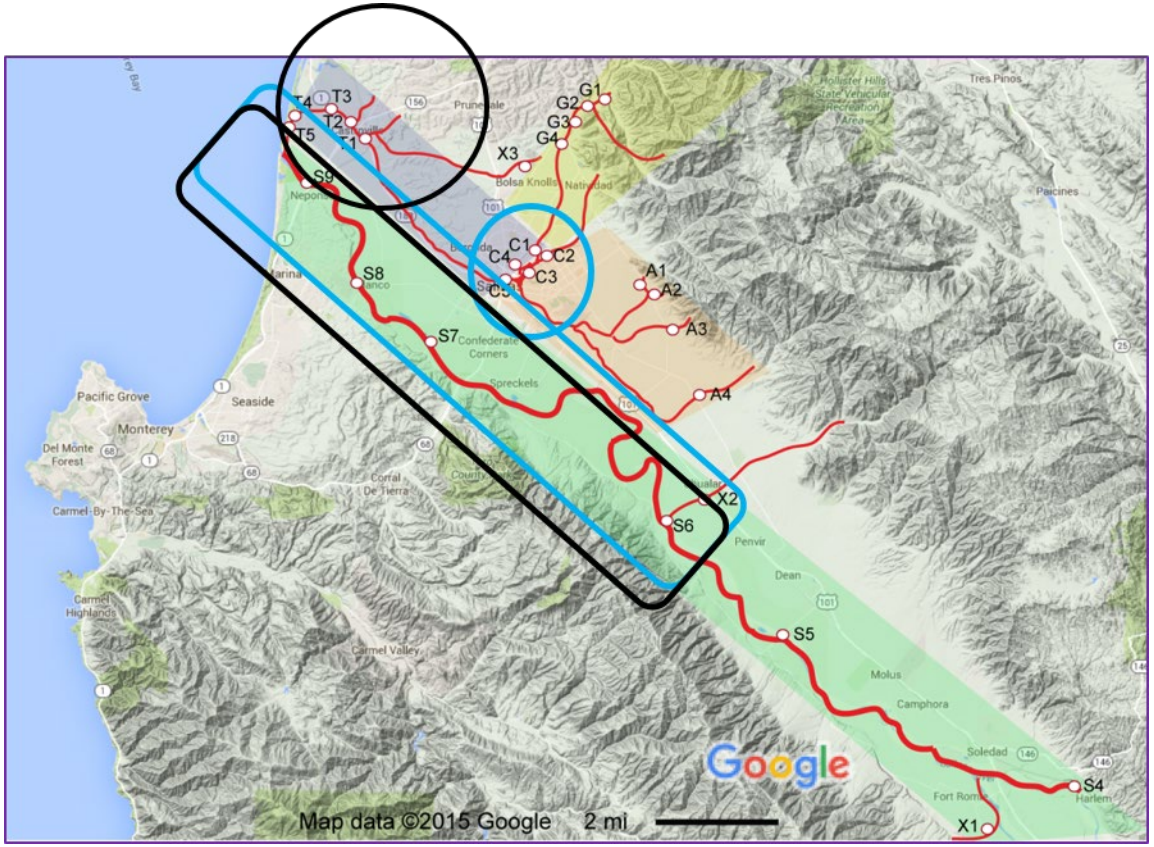
Some *Salmonella* serovars have location preferences

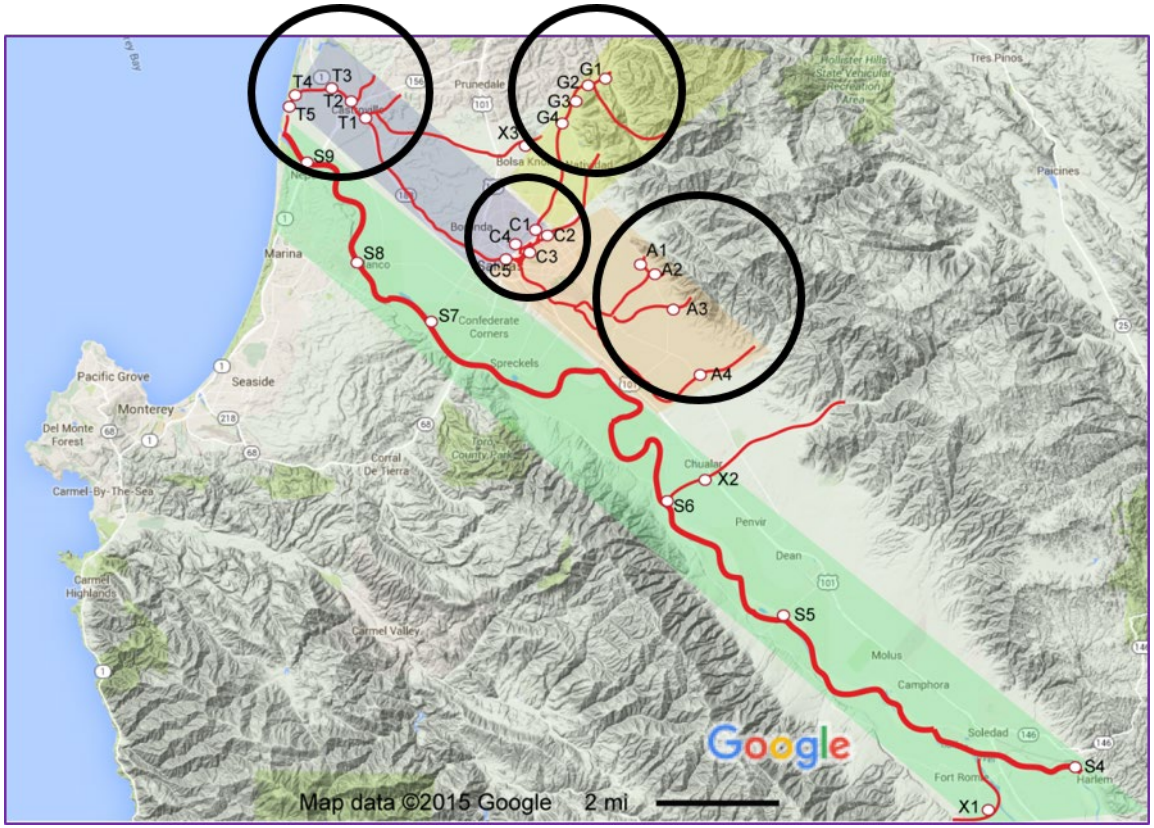


Oranienburg

Montevideo

Salmonella Give is everywhere

Types and Distribution of *L. monocytogenes*



L. monocytogenes located everywhere.
Higher in indicated watersheds.
Many locations near produce fields
Contacted by growers concerning location and sources

1,376 isolates

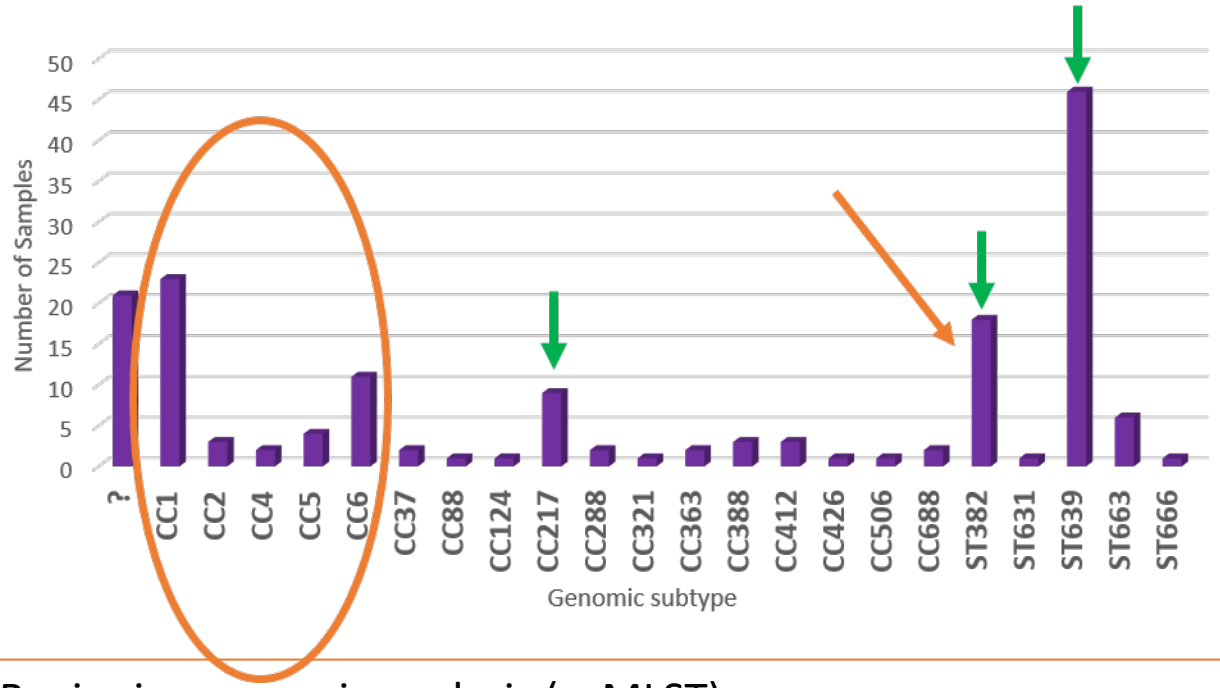
Serotype	Percentage
4b	88%
1/2a	7%
1/2b	6%

<u>*inlA* genotypes</u>
Of 112 isolates: 90% contain intact *inlA* gene
Gorski et al. (2016) PLoS ONE 12:e0167566

All 3 serotypes located everywhere

L. monocytogenes WGS and core genome MLST

In collaboration with Yi Chen (FDA) and S. Kathariou (NC State)



ST382 and ST639 – Higher propensity to be associated with water

CC217 – has been found in environmental sources

--*Lee et al. mBio (2018) 9:e00396-18*

Beginning genomic analysis (cgMLST)
164 isolates (12%)

CC1 (EC1), CC2 (ECIV), CC6 (ECII) – established clonal groups implicated in many outbreaks
CC4 – emerging hypervirulent group first identified in Europe
CC5 – 2014 stonefruit outbreak (1/2b strain)
ST382 – outbreaks: 2014 stonefruit, 2014-2015 caramel apple, 2015-2016 packaged salad


CRIS 050
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