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The Role of Biofilms in Retail Settings

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Estimated annual human health burden of selected known foodborne diseases, United States

Pathogen	Illnesses	Deaths	Case-fatality
Campylobacter	1,322,137	119	0.1%
Salmonella	1,229,007	452	0.5%
E. coli O157:H7	96,534	31	0.5%
L. monocytogenes	1662	266	15.9%

CDC
Centers for Disease Control and Prevention

Scallan, et al., *Emerging Infectious Diseases, 2011*

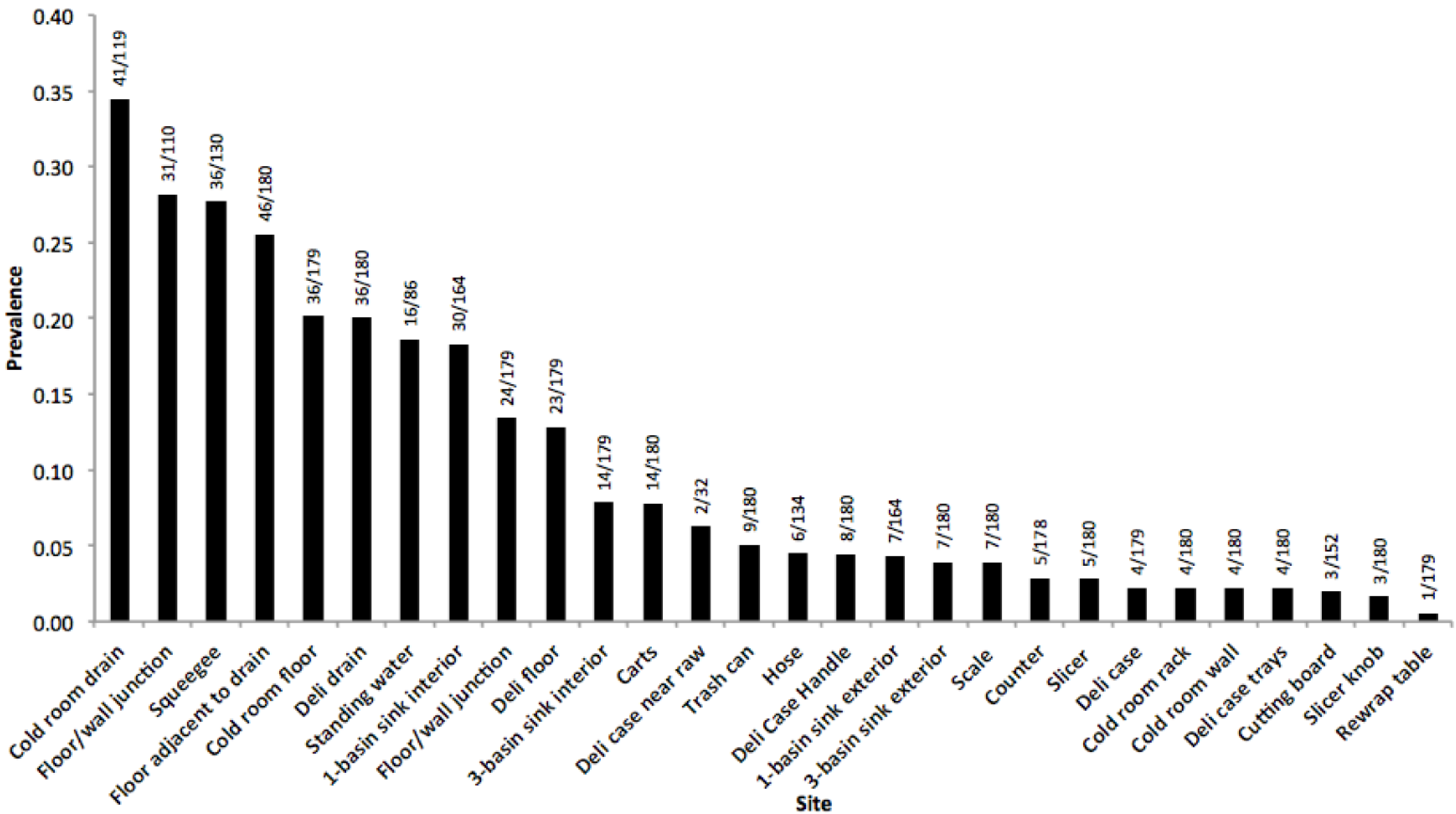
Listeria monocytogenes and Retail: A Challenge

- Ready-to-eat foods: a common source of listeriosis [1]
  - 83% of listeriosis cases from RTE deli meats from deli meats sliced at retail[1]
    - Post-heat treatment contamination[2]
- *L. monocytogenes* prevalence in delis[3]
  - 14.2% of NFCS
  - 4.5% of FCS

Persistent and Transient Strains in Delis

- Strain: unique PFGE pulsotype
- Persistent strain: *L. monocytogenes* with PFGE pattern in the same store for ≥ 3 separate months
- Transient strain: *L. monocytogenes* with PFGE pattern in the same store for < 3 separate months

Prevalence of LM by Site



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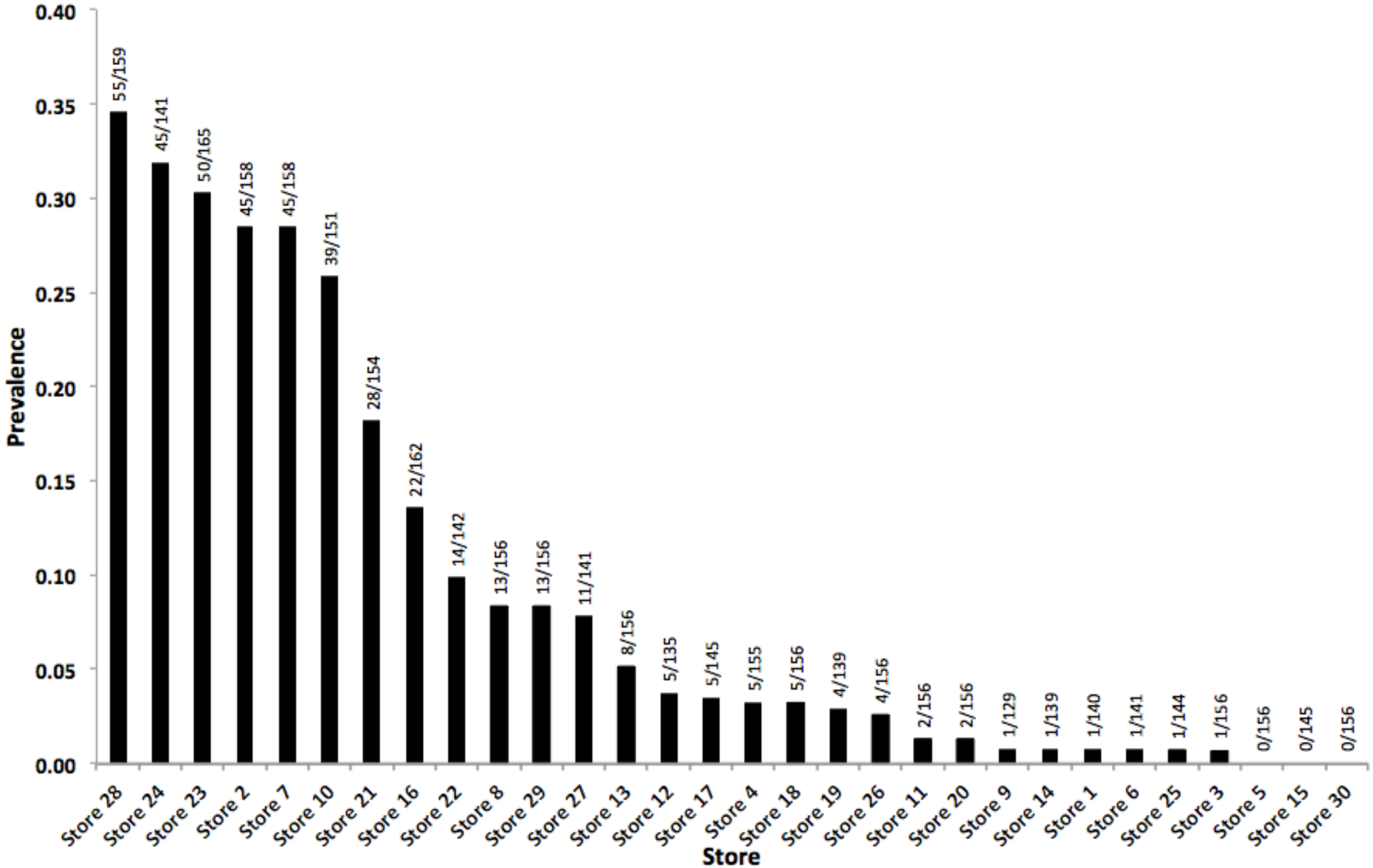
Prevalence of LM by Store



	April	May	June	July	August	September	October	November	December
Food Contact Sites									
Slicer	-	-	-	-	-	-	-	-	-
Deli case	NT	NT	NT	-	-	-	-	-	-
Deli case near raw meat	NT	NT	NT	-	-	-	-	-	-
Deli case trays	NT	NT	NT	-	-	-	-	-	-
3-basin sink interior	NT	NT	NT	CU-57,267	-	-	-	-	-
1-basin sink interior	NT	NT	NT	CU-258,69	-	-	-	CU-294,321	-
Cold room rack	-	-	-	-	-	-	-	-	-
Cutting board	NT	NT	NT	NT	-	-	NT	NT	-
Rewrap table	NT	NT	NT	-	-	-	-	-	-
Counter	NT	NT	NT	-	-	-	-	-	-
Non-food contact sites									
3-basin sink exterior	NT	NT	NT	-	-	-	-	-	-
Floor/wall junction (3-basin)	CU-258,69	CU-258,69	CU-258,69	CU-258,69	CU-258,69	CU-8,96	LM	CU-258,69	CU-258,69
1-basin sink exterior	NT	NT	NT	CU-258,69	-	-	LM	-	CU-258,69
Floor/wall junction (1-basin)	NT	NT	NT	CU-258,69	-	-	LM	CU-258,69	CU-258,69
Deli drain	NT	NT	NT	CU-258,69	CU-258,333	-	CU-258,69	CU-258,69	CU-258,69
Floor adjacent to drain	-	CU-258,69	CU-258,69	CU-258,69	CU-258,69	-	-	CU-258,69	CU-258,69
Deli floor	NT	NT	NT	CU-258,69	-	-	-	CU-258,69	-
Cold room floor	NT	NT	NT	CU-258,69	CU-295,329	-	CU-258,69	CU-258,69	CU-258,69
Cold room wall	CU-258,69	-	-	-	-	-	-	-	-
Cold room drain	NT	NT	NT	CU-258,69	CU-258,69	-	CU-258,69	CU-258,69	CU-258,69
Standing water	NT	NT	NT	NT	-	-	NT	NT	-
Squeegee	NT	NT	NT	CU-258,69	CU-258,69	-	CU-258,69	CU-258,69	CU-258,69
Cart Wheel	-	-	CU-258,69	CU-258,69	-	-	-	-	-
Hose	NT	NT	NT	-	CU-258,69	-	-	-	-
Trash can	-	-	CU-258,69	-	CU-258,69	-	-	-	-
Transfer Points									
Slicer knob	-	-	-	-	-	-	-	-	-
Case handle	-	-	-	-	-	-	-	-	-
Scale	NT	NT	NT	-	-	-	-	-	-

Phenotypic Characteristics of LM

- Biofilms
  - Protect LM against environmental stress[4]
  - Form on various food contact surfaces[5]
  - Potential cause of contamination[6]
  - Influenced by: strains, properties of surfaces, temperatures, growth media, and the presence of other microorganisms[7,8]

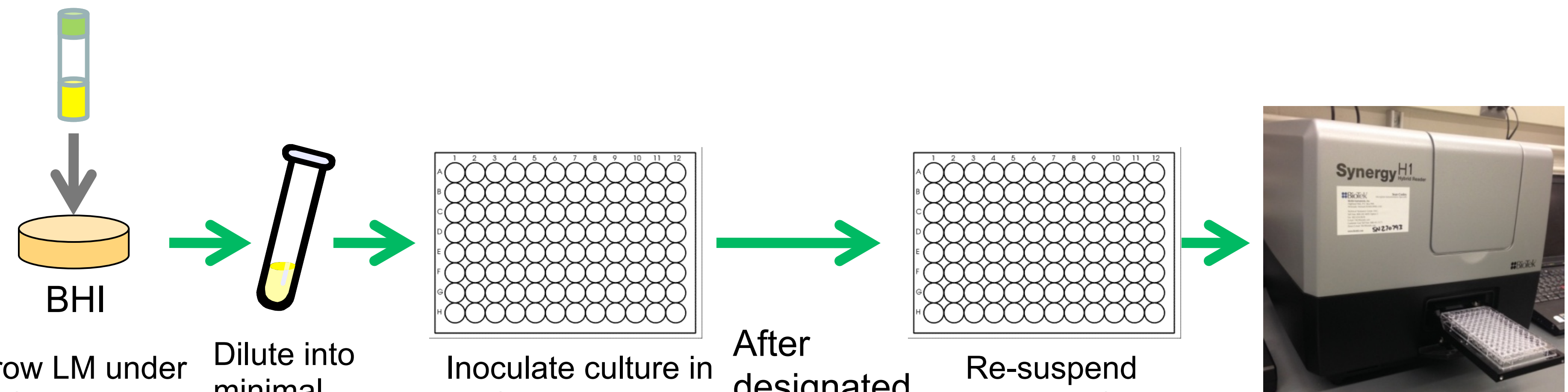
Phenotypic Characteristics of *L. monocytogenes* Retail Isolates

- Hypothesis:
  - *L. monocytogenes* persistent strains are better at forming biofilms
  - There is relationship between biofilm formation and sanitizer tolerance

- Goals of this Work:
  - To assess the ability of 23 persistent strains and 73 transient strains
    - Attachment to abiotic surfaces (indicator of biofilm formation)
    - Sanitizer tolerance
    - Relationship between attachment ability and sanitizer tolerance

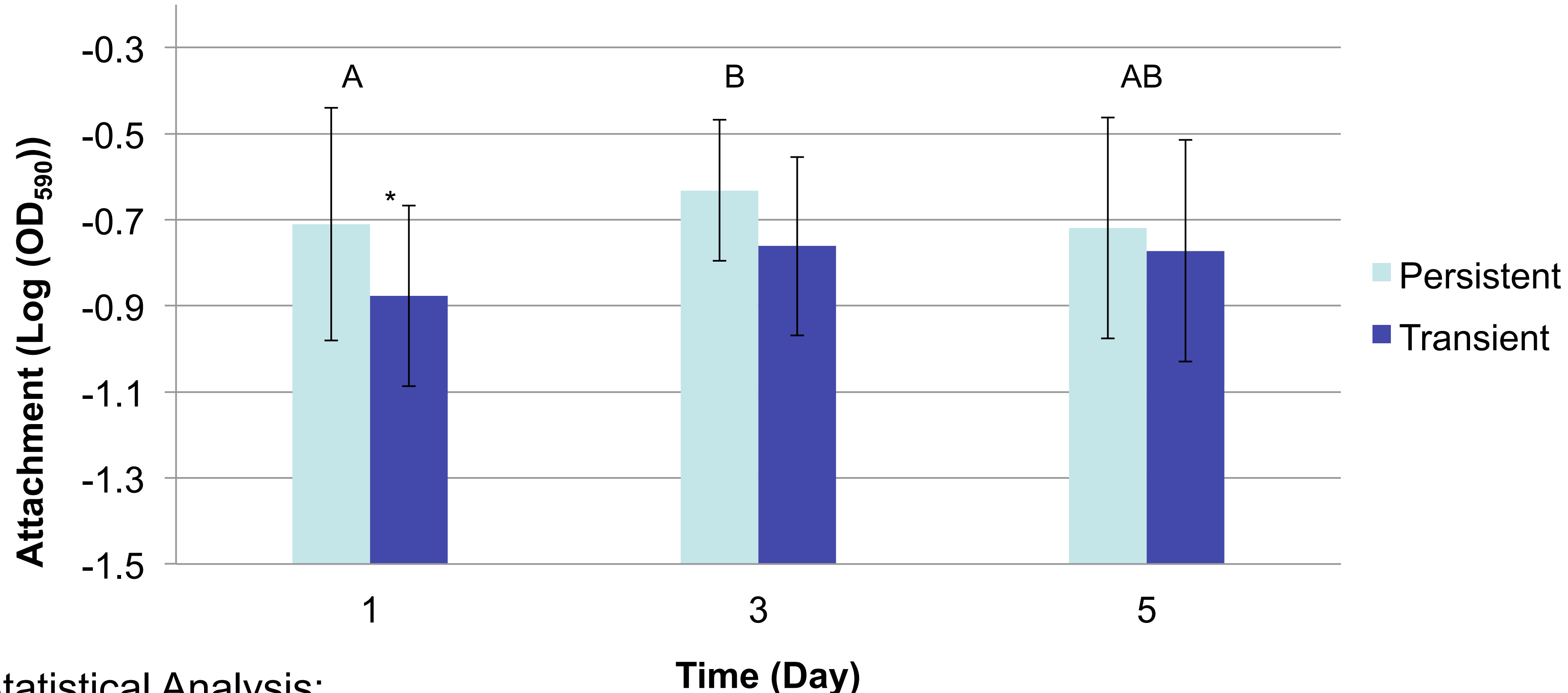
Attachment Assay

Adapted from Lemon et al. 2007, Chen et al. 2013



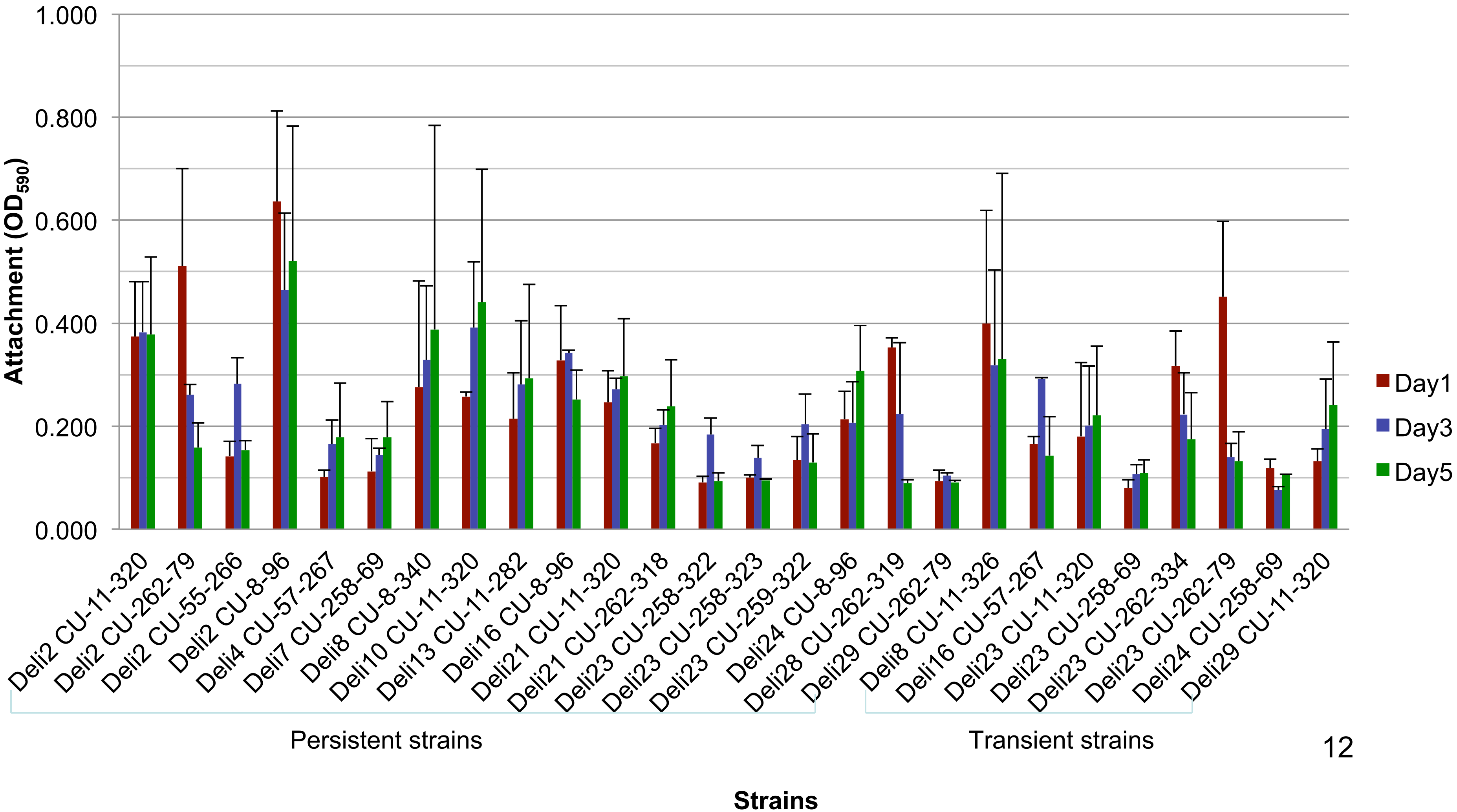
Grow LM under biofilm promoting conditions → Dilute into minimal media (HTM) → Inoculate culture in PVC plates → After designated time (1, 3, or 5 d), wash, stain, and dry biomass → Re-suspend and transfer → Read at OD_{590}

Persistent Strains Attach to Abiotic Surfaces Better than Transient Strains

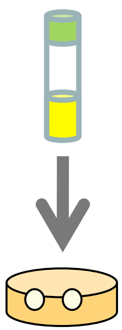


Statistical Analysis:
Generalized linear model (GLM): Log(OD_{590})= “day”, “type”, and “day*type”
LS Means to determine p-value for day*type
* Denotes significance at P<0.05

Isolates Within a Strain Display Significant Variation in Attachment Ability

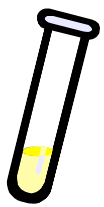


Sanitizer Tolerance Assay

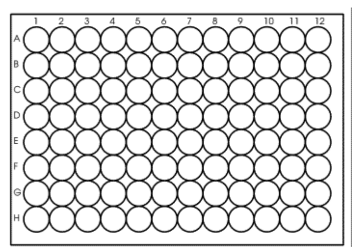


BHI

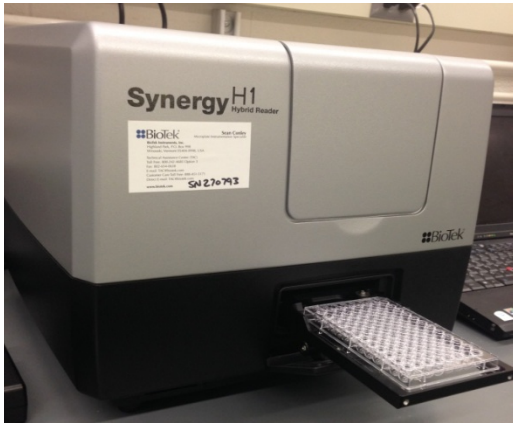
Pick isolated colonies



Suspend in 2X TSB and 1/5XTSB

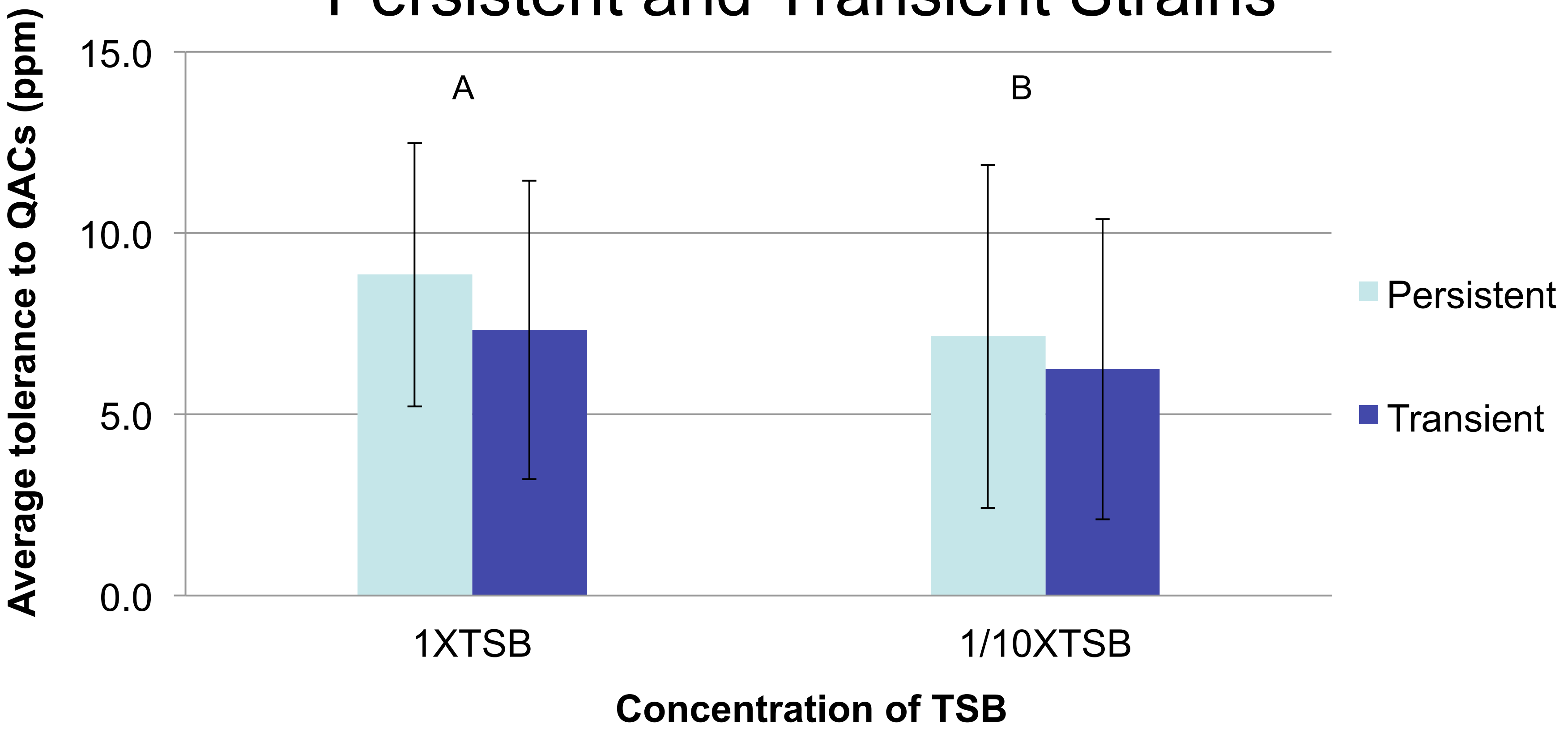


Transfer culture to PVC plate with QACs [6.5 ppm-200ppm]



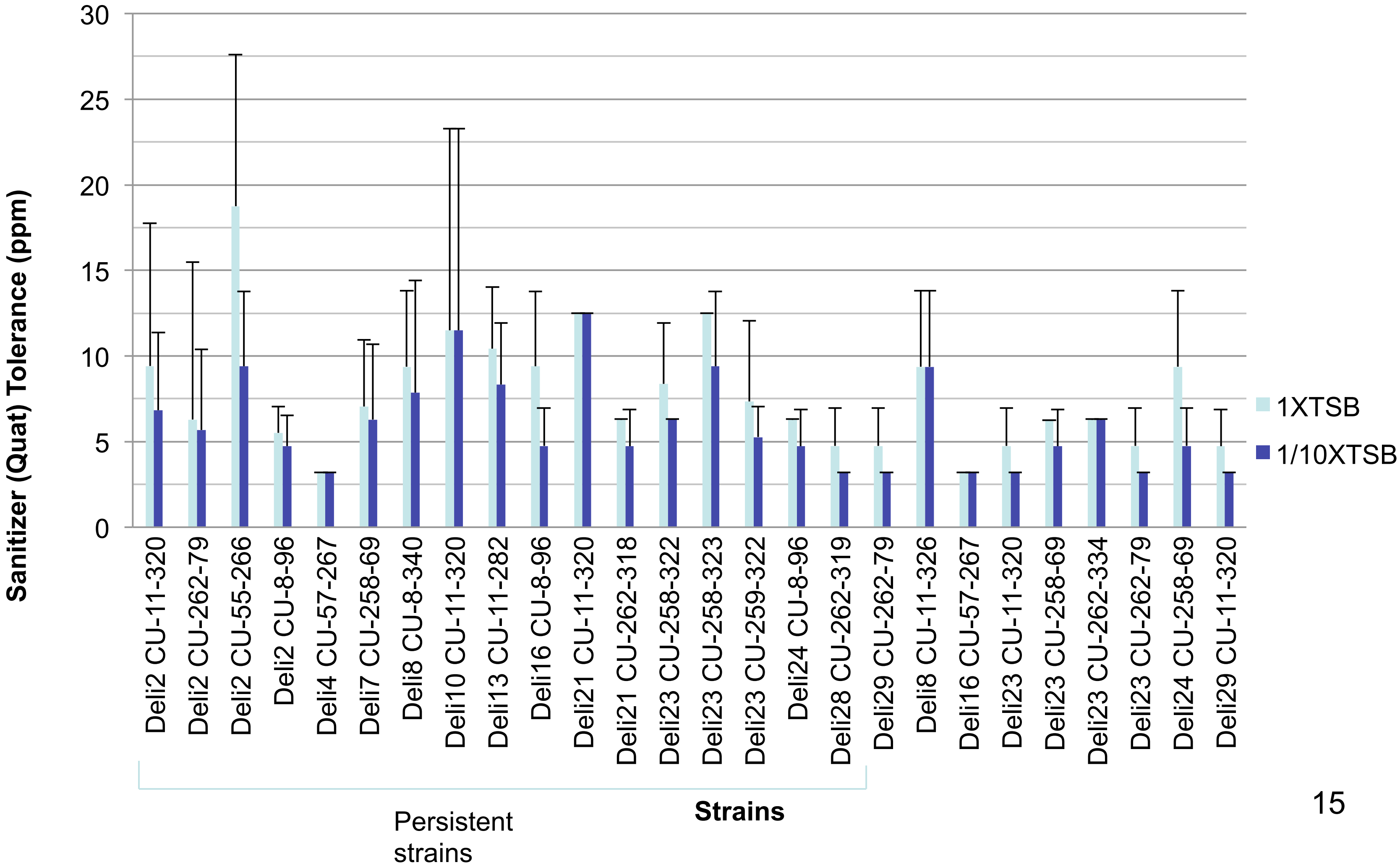
Measure OD_{600} and MICs were determined

No Significant Difference between Persistent and Transient Strains

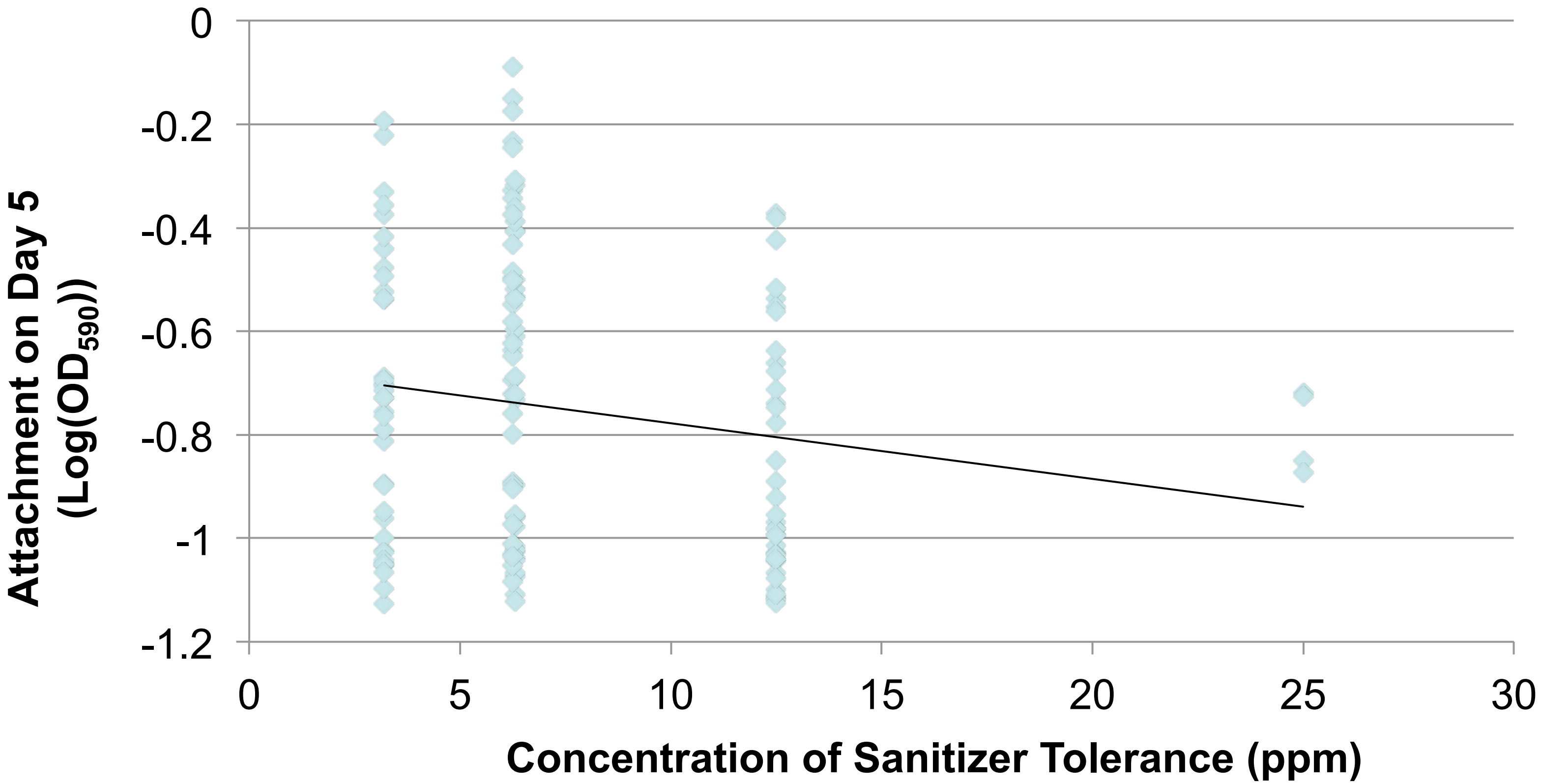


Statistical Analysis:
GLIMMIX: tolerance = “nutrient”, “type”, and “nutrient*type”
LS Means to determine p-value for “nutrient*type”
* Denotes significance at $P<0.05$

Isolates Within a Strain Display Significant Variation in Sanitizer Tolerance



Attachment Ability is Negatively Correlated with Sanitizer Tolerance after Long-term Incubation in Nutrient Rich Conditions



Statistical Analysis:
Linear regression model: Log(OD_{590}) (on different day) = sanitizer tolerance (under different nutrient conditions)
* Denotes significance at P<0.05

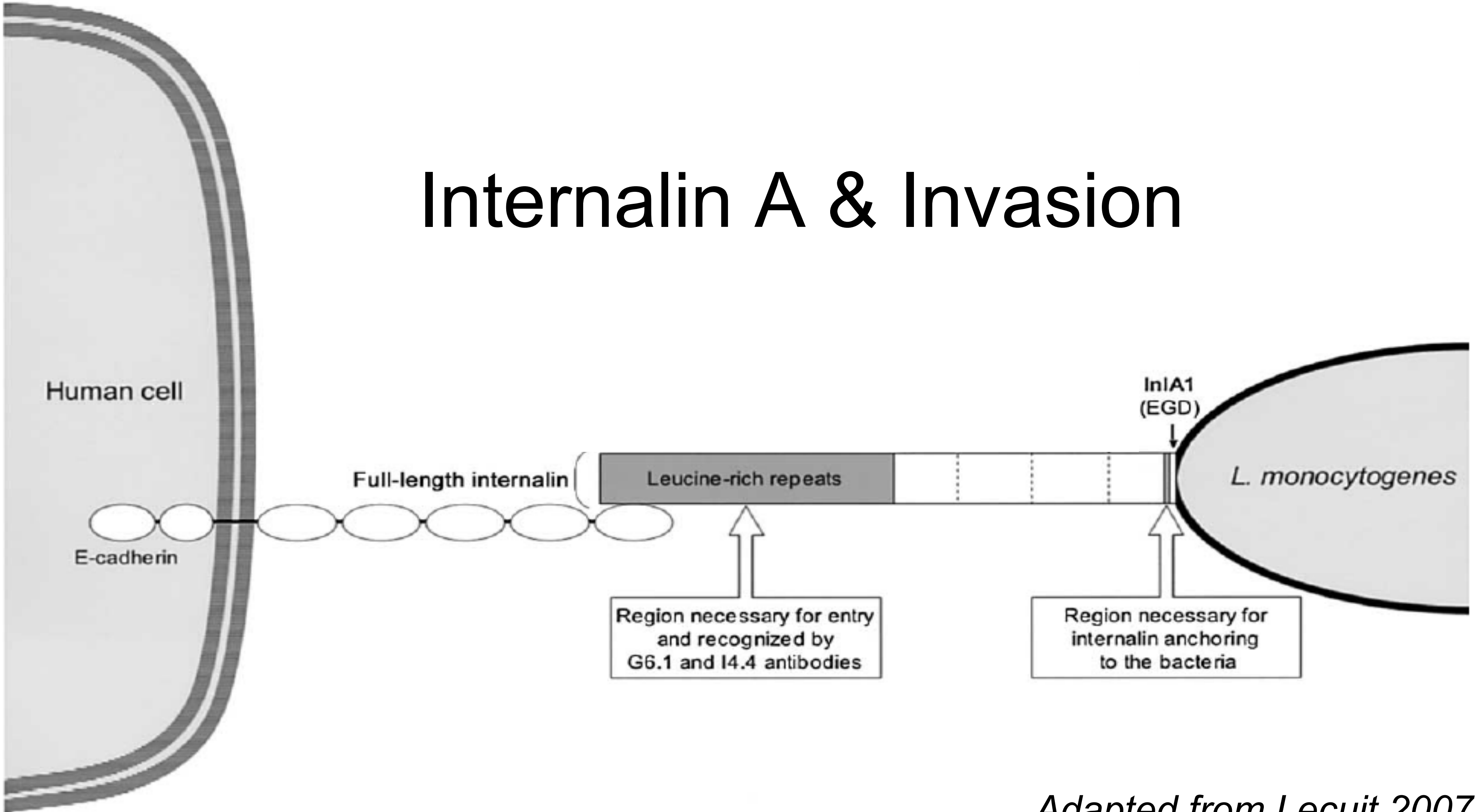
Conclusion

- Persistent LM strains were better at attaching to abiotic surfaces (PVC) than transient strains
- Persistent strains and transient strains displayed similar levels of sanitizer tolerance
  - LM was more tolerant to QACs under nutrient rich conditions than in nutrient limiting conditions
- A negative correlation was found between enhanced cell attachment on day 5 and sanitizer tolerance under nutrient rich conditions
- Both attachment ability and sanitizer tolerance varied widely among isolates of the same strain

Key questions:

- What proportion of isolates in the retail deli environment are virulence attenuated?
  - Food contact surfaces vs non-food contact surfaces?
  - Persistence of virulence attenuated mutants compared to WT strains?

Internalin A & Invasion



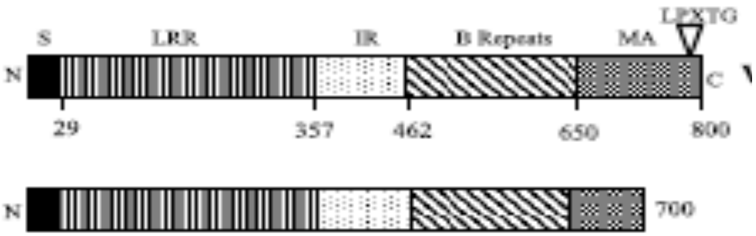
Adapted from Lecuit 2007

Virulence Potential of *L. monocytogenes* from delis

- Up to 45% of isolates from RTE foods have *inlA* premature stop codons
  - Strongly decreased invasiveness of mutant (<10% of WT) in cell culture
  - Rarely found in isolates from clinical cases

WT (EGD-e)

PMSC-3



Van Stelten et al., 2010;
Van Stelten et al., 2008

PMSC Screen

- 981 isolates (Phases I-V)
  - Test for full length *internalin A* vs PMSC
    - 18 known *inlA* PMSCs
    - PCR-based assay:

Gene amplification

↓

Single nucleotide extension PCR

↓

Capillary electrophoresis

Van Stelten et al, 2008;
Van Stelten et al, 2010

inlA PMSCs are Rare Among Deli Isolates

- 15/981 isolates had *inlA* PMSCs
- ~5% of FCS isolates contained PMSCs
  - 8/15 PMSCs associated with FCS isolates
- <1% of NFCS isolates contained PMSCs

inlA PMSCs Not Common in Delis with High *L. monocytogenes* Prevalence

- PMSCs more common in moderate prevalence delis (1-10% average LM+ environmental samples)
  - 11.9% of isolates vs ~2% of isolates from high prevalence stores
  - 8/15 PMSC+ isolates from moderate prevalence delis
- PMSCs significantly associated with FCS isolates from moderate prevalence stores
- **PMSCs always found in transient isolates**

Wang et al., 2015

...mmary of isolates with PMSCs

Store Number[a]	LM Prevalence[b]	Total PMSC[d]	FCS PMSC[f]	NFCS PMSC[h]	PFGE Patterns of PMSC+ isolates[i]	PMSC Locations[j]	PMSC Type[k]
Store 2	High	2	0	2	CU-82-215 CU-341-79	Floor/wall junction under 1-basin sink (NFCS) Cold room drain (NFCS)	PMSC-4 PMSC-3
Store 16	High	1	0	0	CU-100-140	Deli case handle (TP)	PMSC-4
Store 23	High	3	2	1	CU-296-330 CU-82-215	1-basin deli sink interior (FCS) Standing water (NFCS)	PMSC-4
Store 4	Moderate	1	0	1	CU-80-218	Trash cans (NFCS)	PMSC-4
Store 8	Moderate	1	1	0	CU-200-227	1-basin deli sink interior (FCS)	PMSC-4
Store 11	Moderate	1	1	0	CU-200-227	3-basin deli sink interior (FCS)	PMSC-4
Store 17	Moderate	1	0	1	CU-81-215	Trash cans (NFCS)	PMSC-4
Store 19	Moderate	1	1	0	CU-100-140	Countertop (FCS)	PMSC-3
Store 20	Moderate	1	1	0	CU-200-227	3-basin deli sink interior (FCS)	PMSC-4
Store 22	Moderate	2	1	1	CU-180-231 CU-258-332	Deli case (FCS) Deli floor (NFCS)	PMSC-4
Store 13	Low	1	1	0	CU-200-227	Cold room racks (FCS)	PMSC-4
	Total:	**11**	**7**	**4**			

Simmons et al, 2014; Wang et al, (2015)

Persistent Isolates Are More Likely to Form Biofilms and Less Likely to Have *inlA* PMSCs

- Persistent *L. monocytogenes* strains may be better adapted for survival in retail delis through biofilm formation
- Biofilm-forming isolates are less likely to have virulence-attenuating mutations
  - Risk for cross-contamination of surfaces

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