RESEARCH IN PROCESS

Pathogen Physiological State has a Greater Effect on Outcomes of Challenge and Validation Studies than Strain Diversity

SUMMARY

Effective control of foodborne pathogens on produce and in produce-associated environments requires science-based validation of interventions and control strategies. It has previously been shown that strains and/or genetic lineages of a pathogen may differ in their ability to survive different stress conditions. Similarly, the physiological state of bacteria and the conditions under which bacteria are grown also have a considerable impact on their ability to survive produce relevant interventions. This project will assemble a collection of diverse microbes that are appropriate for validation of pathogen interventions in the produce industry, and will determine whether and how exposure to different environmental conditions will affect the ability of these organisms to survive stressful conditions and control strategies. The resulting data, along with the bacterial collection developed as part of this project, will facilitate more reliable identification of effective control strategies that can reduce the risk of foodborne illnesses and pathogen contamination.

OBJECTIVES

1. Assemble a collection of *Salmonella enterica, L. monocytogenes*, Shiga toxin–producing *E. coli*, and relevant surrogate, indicator, and index organisms representing national and international strain diversity associated with produce.
2. Evaluate the strains assembled in Obj. 1 for (i) growth on different produce types and (ii) survival of key produce relevant controls and intervention treatments (e.g., different sanitizers).
3. Expose selected pathogen strains to different environmental and stress conditions (e.g., different growth phases and water activities; pH stress) and evaluate them for subsequent (i) growth on different produce and fruit products and (ii) survival of key produce relevant controls and intervention treatments.
4. Develop and publicize standard protocols for pathogen growth under produce relevant conditions and assemble standard produce pathogen strain sets.

METHODS

Strain collection and genetic characterization. A proposed strain collection including *Salmonella enterica*, *Listeria monocytogenes*, Shiga toxin-producing *E. coli*, and surrogate, indicator and index organisms associated with produce related outbreaks has been evaluated by experts for inclusion in the study. Isolates in the final set will be characterized by whole genome sequencing. Genetically distinct strains of each category will be selected for phenotypic characterization.

Challenge studies. Selected strains will initially be assessed for growth, at different temperatures, on selected produce types previously linked to contamination events, recalls or outbreaks (e.g., tomatoes, leafy greens, cantaloupe). Furthermore, strains' ability to survive stress conditions and intervention treatments (e.g., chlorine wash, peroxyacetic acid, hypochlorite) will be determined. A subset of strains will be selected for further phenotypic characterization to assess growth and survival of different interventions when strains are pre-grown or pre-adapted to different stress conditions.

Table 1. Proposed produce selection and growth conditions

Organism	Produce	Environmental and stress conditions during pre-growth conditions	Planned intervention treatments for all pathogens
Salmonella enterica	Tomatoes, Cucumbers, Romaine Lettuce, Spinach leaves, Cantaloupe, Sprouts	(i) BHI log and stationary phase: 15°C, 22°C (ii) Nutrient limited media [a]: 15°C, 22°C, 37°C (iii) pH stress: pH 5.0 and 7.0 in BHI at 22°C (iv) low water activity: 0.99; 0.85; and 0.70 at 22°C	Chlorine wash (50ppm) Peroxyacetic acid submersion (60ppm) Hypochlorite Cut produce (50ppm) Whole produce (250ppm)
Listeria monocytogenes	Cantaloupe, Spinach leaves, Romaine Lettuce, Sprouts, Apples	(i) BHI log and stationary phase: 7°C, 15°C, 22°C (ii) Nutrient limited media [b]: 7°C, 22°C, 37°C (iii) pH stress: pH 5.0 and 7.0 in BHI at 22°C (iv) low water activity: 0.99; 0.85; and 0.70 at 22°C	
Escherichia coli	Romaine Lettuce, Spinach leaves, Celery, Sprouts	(i) BHI log and stationary phase: 15°C, 22°C (ii) Nutrient limited media [a]: 15°C, 22°C, 37°C (iii) pH stress: pH 5.0 and 7.0 in BHI at 22°C (iv) low water activity: 0.99; 0.85; and 0.70 at 22°C	

[a] M9 minimal salts medium
[b] Chemically defined, minimal medium for *Listeria* with 25mM glucose

RESULTS TO DATE

An initial proposed strain collection was evaluated by experts from academia (6), government (5), and industry (8) who had at least 10 years of experience in food safety. Strains were included when half or more of respondents thought the strains were important; additional selected strains were added based on experts' feedback. Furthermore, strains were selected to ensure inclusion of most common serotypes, lineages or multidrug resistance patterns. The finalized collection is comprised of 20 *Salmonella enterica*, 10 *Listeria monocytogenes* strains, 10 Shiga toxin-producing *E. coli* strains, and 6 surrogate, indicator and index organisms.

BENEFITS TO THE INDUSTRY

After completion of this project, a standardized collection of *Salmonella enterica*, *Listeria monocytogenes*, Shiga toxin-producing *E. coli*, and surrogate, indicator and index organisms that are relevant to produce will be available. Strains will have been characterized genotypically and phenotypically, including for key features relevant to the produce industry (for example, survival of key interventions). In addition, standard operation procedures (SOPs) will be developed for (i) pathogen exposure to different stress conditions and (ii) growth of pathogens under different produce relevant growth conditions. The generated data along with developed SOPs will allow the produce industry to better select strains and to provide a framework for validation, as well as growth and survival studies of specific pathogens in different produce types. This strain collection will be a critical resource that will facilitate development of science-based preventive controls, as required by FSMA.

Table 2. Strain collection after experts' evaluation

Strain	Serotype [Lineage]	Isolation Origin associated with outbreaks
Salmonella enterica	Saintpaul	Jalapeño peppers (2008), multistate US and Canada
	Tennessee	Peanut butter, 2006-7, multistate US
	Typhimurium	Orange Juice, 2005, multistate US
	Poona	Cantaloupe, 2000-2, US and Canada
	Enteritidis - PT30	Almonds, 2000-1, US and Canada
	Javiana	Tomatoes, 2002, multistate US
	Newport (antimicrobial susceptible)	Tomatoes, 2002 and 2005, multistate US
	Newport (multidrug-resistant)	Undercooked ground beef, 2002, multistate US [a]
	Senftenberg 775W	Heat resistant Senftenberg [a]
	Heidelberg	Poultry Producer, 2012-2013, multistate USA
	Typhimurium	Peanut butter, 2008-2009, multistate US
	I 4,[5],12:i:-	Alfalfa Sprouts, 2010-2011, multistate US
	Montevideo	Pistachio nuts, 2009, multistate US
	Litchfield	Cantaloupe, 2009, multistate US
	Poona	Cucumbers, 2015, multistate US
	Anatum	Raw almonds, (Danyluk et al., 2007) [c]
	Infantis	Dry pet food, 2012, multistate US [b]
	Muenchen	Alfalfa Sprouts, Sweetwater Farmers, 2016, Kansas US [b]
	I 13,23:b:-	_ [b]
	Enteritidis	Bean sprouts, 2014, multistate US
Listeria monocytogenes	4b [I]	Coleslaw, human, epidemic, 1981, Canada [d]
	1/2b [I]	Human, epidemic, 1994, Illinois USA [d]
	4d [I]	Human epidemic, coleslaw [d]
	1/2a [II]	Hot dog, human, sporadic, US [d]
	Unknown	Caramel Apple, Dec 2014-2015, multistate US
	Unknown	Packaged Salad Dole, 2016, multistate US
	Unknown	Sprouts from Wholesome, 2014, Illinois, Michigan
	4a [III]	Human sporadic case [d]
	4b [IV]	Animal, goat [d]
	Unknown [II]	Soil, spinach field
Escherichia coli	O26	Chipotle Mexican Grill, 2015, multistate US [e]
	O121	Raw Clover Sprouts, 2014, multistate US [e]
	O157:H7	Baby Spinach, 2006 [c]
	O104:H4	Fenugreek sprouts, 2011, Germany [c]
	O26	Raw Clover Sprouts, 2012, multistate US [e]
	O157	Alfalfa Sprouts, 2016, Minnesota and Wisconsin
	O45	Isolates from human cases [e]
	O111	Cabbage Salad, 2014, Minnesota [e, c]
	O145	Shredded Romaine Lettuce, 2010, multistate US [e, c]
	O103	Isolates from human cases [e]
Surrogate, indicator, and index organisms		
Listeria innocua (FSL C2-0008)	unknown	Fish processing plant, 2000
Escherichia coli (W778)	unknown	Environmental water, plant and soil isolate, (Tomas-Callejas et al., 2011)
Escherichia coli (P149)	unknown	Environmental water, plant and soil isolate, (Tomas-Callejas et al., 2011)
Escherichia coli (S19)	unknown	Environmental water, plant and soil isolate, (Tomas-Callejas et al., 2011)
Escherichia coli (ATCC 700728)	unknown	Naturally occurring non-pathogenic *E. coli*
Enterococcus faecium (ATCC 8459)	unknown	Ropy milk, 1891

[a] Inclusion for validation with interventions
[b] Inclusion to assure 20 most common serotypes
[c] Experts' suggestion
[d] Inclusion to assure serotype diversity
[e] Inclusion of serotypes representing "big six"

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ACKNOWLEDGEMENTS

We thank all experts who provided their feedback, as well as all labs and institutions that provided strains for the study.

LENGTH OF FUNDING

January 1, 2016 – December 31, 2017

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