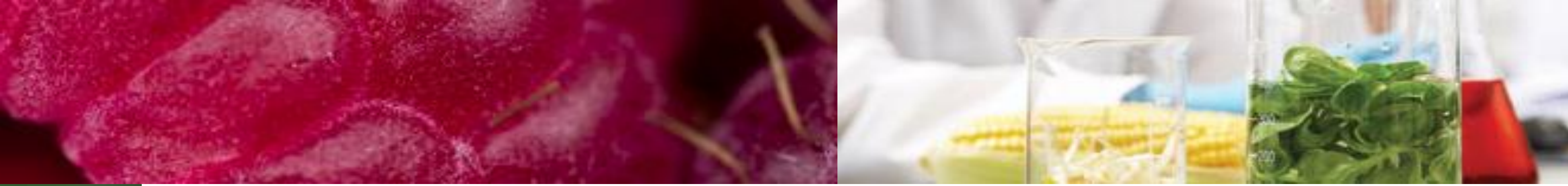

Low Water-Activity Series: Part 1of 4 – Persistent Strains and Source Tracking Principles in Low aw Food Processing Environments

Moderator: Joshua Gurtler, USDA-ARS, United States

Sponsored by the IAFP FOUNDATION

Please consider making a contribution

This webinar is being recorded and will be available to IAFP members within one week.

Today's Participants

Jeffrey Lee Kornacki, Ph.D.
Kornacki Microbiological Solutions, United States

Dr. Kornacki is an industrial forensic food microbiologist. He has assisted and continues to assist many companies during environmental and product contamination concerns including FDA and USDA recalls, and has made well over 850 troubleshooting related plant visits across a vast assortment of food processing industries in his career. He is an active member of IAFP and several PDGs including the Low Moisture Foods PDG. He received the IAFP Sanitarian award (2010), its Wisconsin chapter's (WAFP) Laboratorian of the Year award (2010) and is past Chairman of IAFP's Food & Hygiene PDG from 2011 to 2013. He became an IAFP Fellow in 2017 and has published on a wide variety of food microbiology topics.

Joshua Gurtler
USDA-ARS, United States

Joshua Gurtler is a Research Scientist at the USDA/ARS in Wyndmoor, PA, where he has worked for 14 years. Dr. Gurtler's current work involves interventions for the inactivation of foodborne pathogens in fresh produce, soil, water, compost, and dried foods. Joshua has published numerous peer-reviewed scientific manuscripts, 9 book chapters, is an editor of three books and author of one patent and one patent that is pending. He has served as a member or chair on several IAFP committees. Dr. Gurtler is a co-scientific editor for the Journal of Food Protection. He has delivered numerous scientific research presentations, including invited presentations, in the U.S., Canada, China and Korea. He resides with his wife and three children in Phoenixville, PA.

IAFP Low Water Activity Webinar Series

Persistent Strains and Source Tracking Principles in Low a_w Food Processing Environments

By:

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March 29, 2021

"Bacteria, by any reasonable criterion, were

in the beginning, are now, and ever shall be, the most successful organisms on earth."

Stephen Jay Gould,

Full House: The spread of excellence from Plato to Darwin, 1996.

Estimated Annual Foodborne Disease From *Selected* bacterial pathogens in United States*

Bacterium	No. Total Illnesses*	*% Total Illnesses**	% Hos-pitalized	*% Deaths*	No. Deaths
Salmonella (non-typhoidal)	1.0 M	11	27.2	0.5	378
Clostridium perfringens	970,000	10	0.6	<0.1	9
Staphylococcus	241,000	2.6	6.4	<0.1	6
STECS	176,000	1.9	59.0	0.8	20
Yersinia enterocolitica	98,000	1.0	34.4	2.0	29
B. cereus	63,400	0.7	0.4	0	0
ETEC	18,000	0.20	0.8	0	0
Listeria monocytogenes	1600	<0.02	94	15.9	255

*Among 31 most common foodborne pathogens causing 9.4 million estimated illnesses in the United States; Scallan, et al. 2011. Foodborne Illness Acquired in the United States – Major Pathogens. Emerg. Infect. Dis. 17(1):7-15.

Persistent Vs. Transient Strains

Persistent strains are those specific molecular subtypes which are repeatedly isolated "in the same factory during an extended period of time; typically months or years."

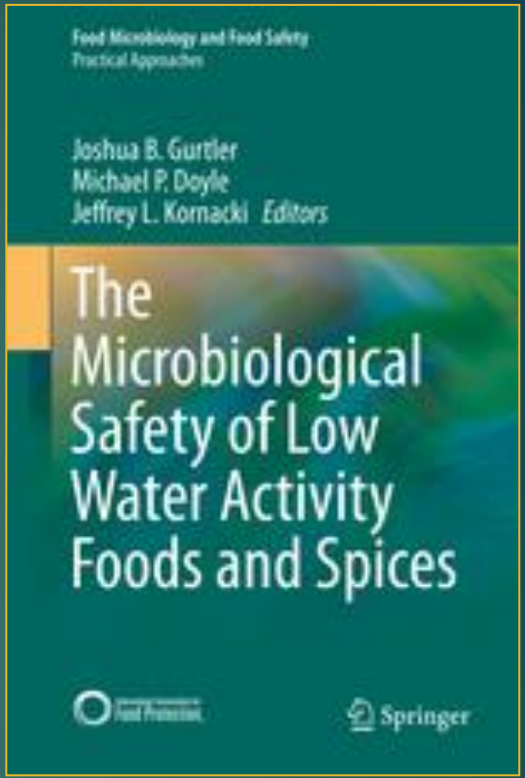
"Hence, the persistent strains may

cause repeated product contamination[1]"

- *[1]Kastberg, V. G. and L. Gram. 2009. Model systems allowing quantification of sensitivity to disinfectants and comparison of disinfectant susceptibility of persistent and presumed non-persistent Listeria monocytogenes. J. Appl. Microbiol. 196:1667-1681.*

Synonymous Terms

- Persistent strains
- Endemic strains
- "Systemic" strains
- House bugs/pets
- Recurrent strains



https://www.springer.com/us/book/9781493920617

Kornacki, J. L. 2014. Processing plant investigations: Practical approaches to determining sources of persistent bacterial strains in the industrial food processing environment. In, J. B. Gurtler, M. P. Doyle and J. Kornacki (Eds.), *The Microbiological Safety of Low Water Activity Foods and Spices*. Springer, New York. Pp. 67-83.

Strain Persistence

How common is this phenomenon?- VERY!

The *Salmonella* experience –

"*Salmonella* can persist for long periods of time in the dry state and in low-moisture products[1]"

2463 different serotypes[2]

32 years active troubleshooting

>850 facility visits (about 2/3 *Salmonella* related)

One serotype for years, *even decades* in many dry food processing facilities visited *in which a Salmonella strain or strains have previously been isolated*

[1]Scott, et al, 2009. Control of *Salmonella* in low-moisture foods I: Minimizing entry of *Salmonella* into a processing facility. Food Protection Trends. June. Pp. 342-353.

[2]Brenner, et al. 2000. *Salmonella* nomenclature. J. Clin. Microbiol. 38(7): 2465-2467.

Transient vs. Persistent

- Transient strain
  - Enters the facility but does not stay
    - Programs eliminate organism
    - Unable to adapt and replicate
- Persistent strains
  - Enters the facility and stays
    - Programs fail to eliminate organism
    - Establishes niche(s)
  - Surface chemistry (e.g., serotype) and/or genetic discriminatory testing

Examples of *Listeria monocytogenes* strain persistence for years*

Food	Years	Country	Serotype
Cheese	4	Switzerland	4b
Cheese, blue veined	7	Sweden	3b
Fish smoked	1.2 (14 months)	Finland	1/2a and 4b
Ice cream	7	Finland	1/2
Meat, sliced luncheon	4	Norway	Not determine d
Mussels, smoked	3	New Zealand	1/2
Pâté	2*	UK	4b(x), 4b
Poultry, cooked	1	Ireland	1/2
Poultry, cooked deli products	12	United States	4b
Salmon, cold smoked	4	Denmark	ND
Trout, smoked/salmon, gravad	>4	Sweden	1/2a

*Excerpts from: Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. J. Food Prot. 65(4):709-725.
**Product from one plant was source of an outbreak from 1987 to mid 1989.

Other Examples of Persistence

Same *Salmonella* serotype across 10 years in cereal plant environment

Journal of Food Protection, Vol. 76, No. 2, 2013, Pages 227–230
doi:10.4315/0362-028X.JFP-12-209

A Recurrent, Multistate Outbreak of *Salmonella* Serotype Agona Infections Associated with Dry, Unsweetened Cereal Consumption, United States, 2008†

ELIZABETH T. RUSSO,[1,2*] GWEN BIGGERSTAFF,[2] R. MICHAEL HOEKSTRA,[2] STEPHANIE MEYER,[3] NEHAL PATEL,[2] BENJAMIN MILLER,[4] AND ROB QUICK,[2] FOR THE *SALMONELLA* AGONA OUTBREAK INVESTIGATION TEAM[5]

Personal Observation – *Salmonella* persistence over 20 years a few plants

Same *Listeria* across 12 years: RTE Meat

Short-term genome evolution of *Listeria monocytogenes* in a non-controlled environment

Renato H Orsi[1], Mark L Borowsky[2,7], Peter Lauer[3], Sarah K Young[2], Chad Nusbaum[2], James E Galagan[2,4], Bruce W Birren[2], Reid A Ivy[1], Qi Sun[5], Lewis M Graves[6], Bala Swaminathan[6] and Martin Wiedmann*[1]

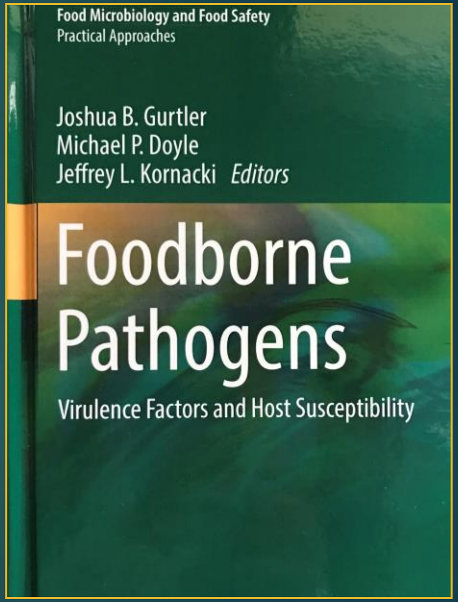
BMS Genomics. 2008. 9:539

Biofilms

"Growth on surfaces offers numerous advantages to microorganisms and therefore biofilms are the predominant growth form of microorganisms in natural environments[1]"

"Failure to clean and effectively sanitize a microbial growth niche site will likely lead to development of a biofilm at that site that may be many orders of magnitude more resistant to destruction by sanitizers[2]"

"…biofilms and polysaccharide capsules … facilitate persistence of pathogens in the processing environment…" [3]



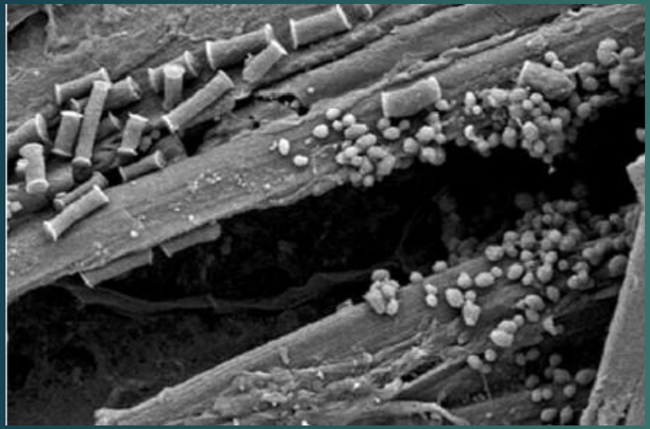
https://www.springer.com/us/book/9783319568348

[1]**Joseph Frank, Professor, Food Science Department and Center for Food Safety, University of Georgia (personal communication).**

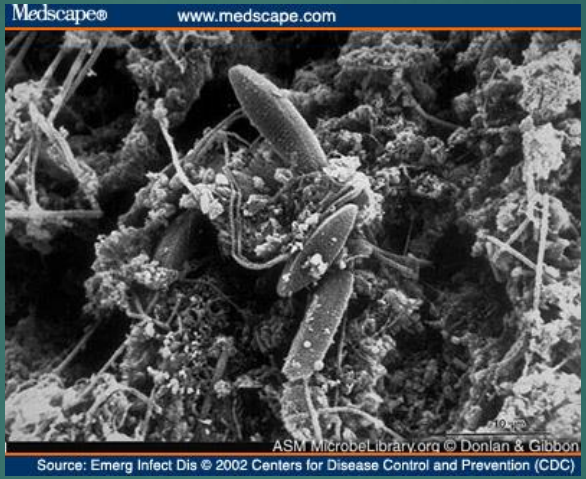
[2]***Kornacki, J. L.*** **How do I sample the environment and equipment?** ***Chapter 7. In, J. L. Kornacki (ed.), Principles of Microbiological Troubleshooting in the Industrial Food Processing Environment, Springer. New York. Pp. 125-136.***

[3]**Venkitanarayanan, et al. 2017. The effects of environmental conditions and external treatments on virulence of foodborne pathogenes. In, Gurtler, J. B., M.P. Doyle and J.L. Kornacki.** ***Foodborne Pathogens:Virulence Factors and Host Susceptibility*****. Springer, New York. Pp. 305-319.**

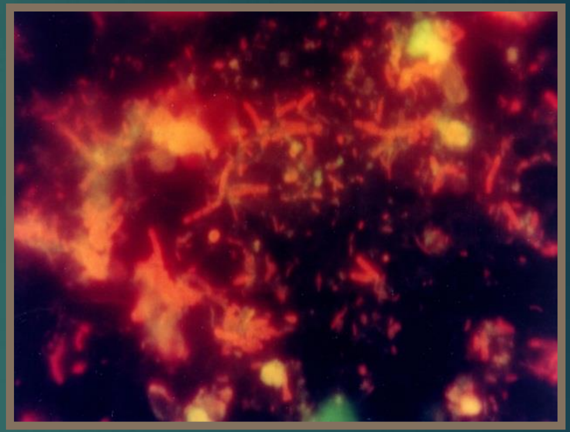
Native Biofilms – Some Examples



Ripening Shelves During Cheese Manufacture[1]



-Steel Surface in Industrial Water System[2]



Acridine Orange Stained Biofilm- Catch Pan Below Condensor Unit– RTE Meat Plant[3]

[1]Mariani, et. al. 2007. Biofilm ecology of wooden shelves used in ripening the french raw milk smear cheese Reblochon de Savoie. J. Dairy Sci. 90:1653-1661.

[2]Donlan, R. M. Biofilms: Microbial life on surfaces. See http://bcbsma.medscape.com/viewarticle/441355_print

[3]Slide Provided Courtesy of Dr. Amy Wong, Food Research Institute, Madison, WI July, 2008

Biofilms: Advantages to the Organism

Access to nutrients under low nutrient conditions that predominate in nature

Protection from environmental or host stress

Ease of genetic exchange

Jefferson, K. K. 2004. What drives bacteria to produce a biofilm? FEMS Microbiol. Ltr. 236:163-173

Sanitizer and Heat Resistance: "*What Doesn't Kill me, makes me stronger*"

Biofilm sanitizer resistance: Widely reported as up to 1000x greater

Listeria monocytogenes

- Planktonic cells vs biofilms:

Pasteurization: (wet heat): 71.7°F (161°F) 15 seconds: 3.6 to 5.2 $\log_{10}$ reduction of *Listeria monocytogenes*[1]

Listeria monocytogenes biofilm: 40 minutes at 70°C (158°F)[2] (wet heat): (>160X more heat resistant in a biofilm)

[1]Mackey, B. M. and N. Bratchell. 1989. A review: The heat resistance of *Listeria monocytogenes*. Lett. Appl. Microbiol. 9:89-94.

[2]Chmielewski, R. A. N. and J. F. Frank. 2004. A predictive model for heat inactivation of *Listeria monocytogenes* biofilm on stainless steel. J. Food Prot. 67(12):2712-2718.

Evicting and Preventing Development of Persistent strains

1. *Find them or the conditions that favor microbial growth*
2. *Eliminate the conditions that favor their development (Do's and Don'ts)*
3. Eliminate the organisms

Principal Source of Microbial Contamination in Processed Foods: *Processing Environment*

"… *cross contamination* …was mentioned as the *most important factor* relating to the presence of pathogens in prepared foods"[1]

Environmental contamination is the principle source of contamination of processed foods

It is from the post-processing (post-CCPm) environment[2,3,4,5]

[1]Reij and E. D. Den Aantrekker. 2004. Recontamination as a source of pathogens in processed foods. Int. J. Food Microbiol. 91:1-11.
[2]Allan, J.T., J.L. Kornacki, Z. Yan, and L.L. Genzlinger. 2004. Temperature and Soil Effects on the Survival of Selected Foodborne Pathogens on a Mortar Surface. Journal of Food Protection. J. Food Prot. 67(12):2661-2665.
[3]Kornacki, J.L. 2009. The missing element in microbiological food safety inspection approaches, Part 1. February-March, Food Safety Magazine.
[4]Kornacki, J.L. 2009. The missing element in microbiological food safety inspection approaches, Part 2. April-May, Food Safety Magazine.
[5]Behling, R. G. 2010. Selected pathogens of concern to industrial food processors: Infectious, toxigenic, toxico-infectious, selected emerging pathogenic bacteria., Chapter 2. *In*, Kornacki, J. L. (ed.), *Principles of Microbiological Troubleshooting in the Industrial Food Processing Environment*, Springer, New York. p.5-61

Examples of Outbreaks Attributed to Environmental Contamination

Product	Pathogen	Comment	Reference
Ice Cream	***S.*** **Enteritidis**	**Pasteurized ice cream mix in tanker truck previously used for transporting raw liquid eggs**	**Hennessy *et al.* (1996)**
Infant formulae	***S.*** **Ealing**	**Contamination from the processing environment, insulation material of the drying tower**	**Rowe *et al.* (1987)**
Soft cheese	***S.*** **Berta**	**Cheese ripening in buckets previously used for chicken carcasses**	**Ellis *et al.* (1998)**
Cooked sliced ham	***S.*Typhimurium**	**Cooked ham placed into containers previously used for curing raw pork**	**Llewellyn *et al.* (1998)**
Chocolate	**S. Napoli**	**Possibly contaminated water used in double-walled pipes, tanks,**	**Gill *et al.* (1983)**
Chocolate	***S.*** **Eastbourne**	**Contamination from the processing environment**	**Craven *et al.* (1975)**
Butter	***S.*** **Eastbourne**	**Contamination from the processing environment**	**Lyytikainen *et al.* (2000)**
Hot dogs	***L. monocytogenes***	**Contamination from the processing environment**	**Anonymous (1999)**
Canned salmon	***C. botulinum***	**Contamination from the processing environment, cooling water**	**Anonymous (1984);Stersky *et al.* (1980)**
Lasagna	***S. aureus***	**Growth of *S. aureus* in the processing equipment, improper cleaning**	**Woolaway *et al.* (1986);Aureli *et al.* (1987)**

Examples of Outbreaks Attributed to Environmental Contamination *continued*

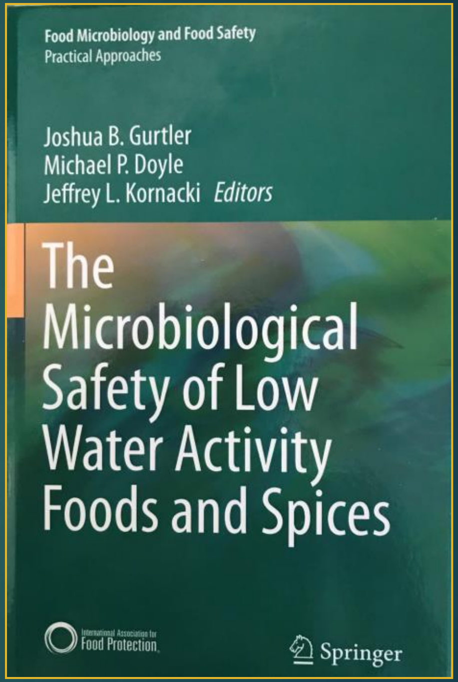
Product	Pathogen	Comment	Reference
Different foods	*E. coli* O157:H7	Contaminated meat grinder and equipment at retail level	Banatvala *et al.* (1996)
Chocolate milk	*Y. enterocolitica*	Probably during manual mixing of pasteurization milk and chocolate or contaminated chocolate syrup	Black *et al.* (1978)
Canned meat	*S.* Typhi	Use of non-potable water for can cooling	Ash *et al.* (1964); Stersky *et al.* (1980)
Crabmeat	*S. aureus*	Contamination during manual picking of cooked meat	Bryan (1980)
Canned mushrooms	*S. aureus*	Possible growth of *S. aureus* in the brine bath before canning	Hardt-English *et al.* (1990)
Flavored Yogurt	*E. Coli* O157:H7	Pump previously used for raw milk	Morgan *et al.* (1993)
Pastry	*S.* Enteritidis PT4	Equipment previously used for raw eggs or insufficiently cleaned piping and nozzles used for cream	Evans *et al.* (1996)
Yeasts	*S.* Műnchen	Contamination from the processing environment	Joseph *et al.* (1991
Pasteurized milk	*S.* Typhimurium	Possibly cross-connection between raw and pasteurized milk	Lecos *(1986)*
Pasteurized milk	*E. coli* O157:H7	Contamination from pipes and rubber seals of the bottling	Upton & Coia (1994)
Mexican type cheese	*L. monocytogenes*	Contamination from the processing environment	Linnan *et al.* (1988)

Behling, et al. 2010. Selected pathogens of concern to industrial food processors: Infectious, toxigenic, toxico-infectious, selected emerging pathogenic bacteria, Chapter 2. *In*, Kornacki, J. L. (Ed.), Principles of Micorbiological Troubleshooting in the Industrial Food Processing Environment, Springer, New York. p.2.

Microorganisms Associated with Foodborne Illness 2007-2012 World- Wide and Recalls

***Salmonella* (94% of US <u>low water activity</u> food recalls and 53% of outbreaks world-wide)**

Shiga-Toxin Producing *Escherichia coli* (STEC)
Bacillus cereus
C. perfringens
C. botulinum
Staphylococcus aureus
***Cronobacter spp.* (formerly *E. sakazakii*)**
<u>Listeria monocytogenes (recall only)</u>



7315 cases of bacterial infection
63 deaths

Santillana, S. M., and J. F. Frank. 2014. Challenges in the control of foodborne pathogens in low-water activity foods and spices, Chapter 2. In, J. B. Gurtler, M. P. Doyle and J. L. Kornacki (Eds.), The Microbiological Safety of Low Water Activity Foods and Spices. Springer, New York. Pp. 15-34.

Low a_w Foods Implicated in Outbreaks 2007-2012 World-Wide

Rice (including Imperial, fried, Spanish, rice cereal)
Rice and corn snack
Seeds including Turkish Pine Nuts
Nuts
Almonds, raw shelled walnuts, in-shell hazelnuts, peanut butter, roasted pistochios

Herbs & Spices
White ground pepper, black pepper in salami
Dry pet food
Dry milk
Dried tofu
Infant formula
Sweets and chocolate

Santillana, S. M., and J. F. Frank. 2014. Challenges in the control of foodborne pathogens in low-water activity foods and spices, Chapter 2. In, J. B. Gurtler, M. P. Doyle and J. L. Kornacki (Eds.), *The Microbiological Safety of Low Water Activity Foods and Spices*. Springer, New York. Pp. 15-34.

U.S. Recalls Low a_w Foods 2007-2012: No lethality treament

Salmonella	*E. coli* O157:H7
Black, white and red pepper	Organic cacao nibs
Curry spice	
Garlic powder	Hazelnut and mixed nuts
Nuts, hazelnuts (shelled and unshelled, raw kernels),	
walnuts, pistachio kernel products	
Nutmeg	
Organic celery seed	
Parsley powder	
Peppermint organic tea	
Sesame seeds, pine nuts	
Soybean flour and soy meal	
Spice packages, seasonings, blends, seasoning salt	
spice rub, gravy mix, onion dip mix, soup mix,	
sauce mix four cheese risotto mix	

Santillana, S. M., and J. F. Frank. 2014. Challenges in the control of foodborne pathogens in low-water activity foods and spices, Chapter 2. In, J. B. Gurtler, M. P. Doyle and J. L. Kornacki (Eds.), *The Microbiological Safety of Low Water Activity Foods and Spices*. Springer, New York. Pp. 15-34.

U.S. Recalls 2007-2012: Processed Low a_w Products

Salmonella	*L. monocytogenes*	*C. botulinum*
Crushed roasted Thai **red pepper**	Popcorn with flavors	Chai concentrate
Dry **pet foods** including dog and cat food, flake fish food	Spreads	
Peanut butter	Salsa	
Snack products with chili, also corn chips, corn sticks , potato chips, snack mixes (including with cashews), potato crisps, potato chips (including with barbeque sauce), crackers	Italian sausage	
Snack mixes,including with Cashews, pretzels, mixes with pretzels	Dips	
Egg noodle	Peanut butter	
Pancake, cake, cookie mix, batter mix	Cheese	
Spreads, cheese ball mix		
Dry roasted hazlenut kernels		
Chocolate covered peanuts, white chocolate baking squares		
Oatmeal, instant variety pack, and with brown sugare		
Protein powders, NFDM, dry whey, sweet dairy whey powder, prebiotic powder formual		

Santillana, S. M., and J. F. Frank. 2014. Challenges in the control of foodborne pathogens in low-water activity foods and spices, Chapter 2. In, J. B. Gurtler, M. P. Doyle and J. L. Kornacki (Eds.), The Microbiological Safety of Low Water Activity Foods and Spices. Springer, New York. Pp. 15-34.

The Coming Storm- Food Safety Magazine

SPICES By Jeffrey L. Kornacki, Ph.D.

The Coming Storm in the Spice Industry

Kornacki, J.L. 2016. The Coming Storm in the Spice Industry. Food Safety Magazine. December 2016/January 2017.

The Coming Storm in the Spice Industry. Part II. What the Industry Can Do. Food Safety Magazine. February/March 2017.

1. Find them or the conditions that favor microbial growth

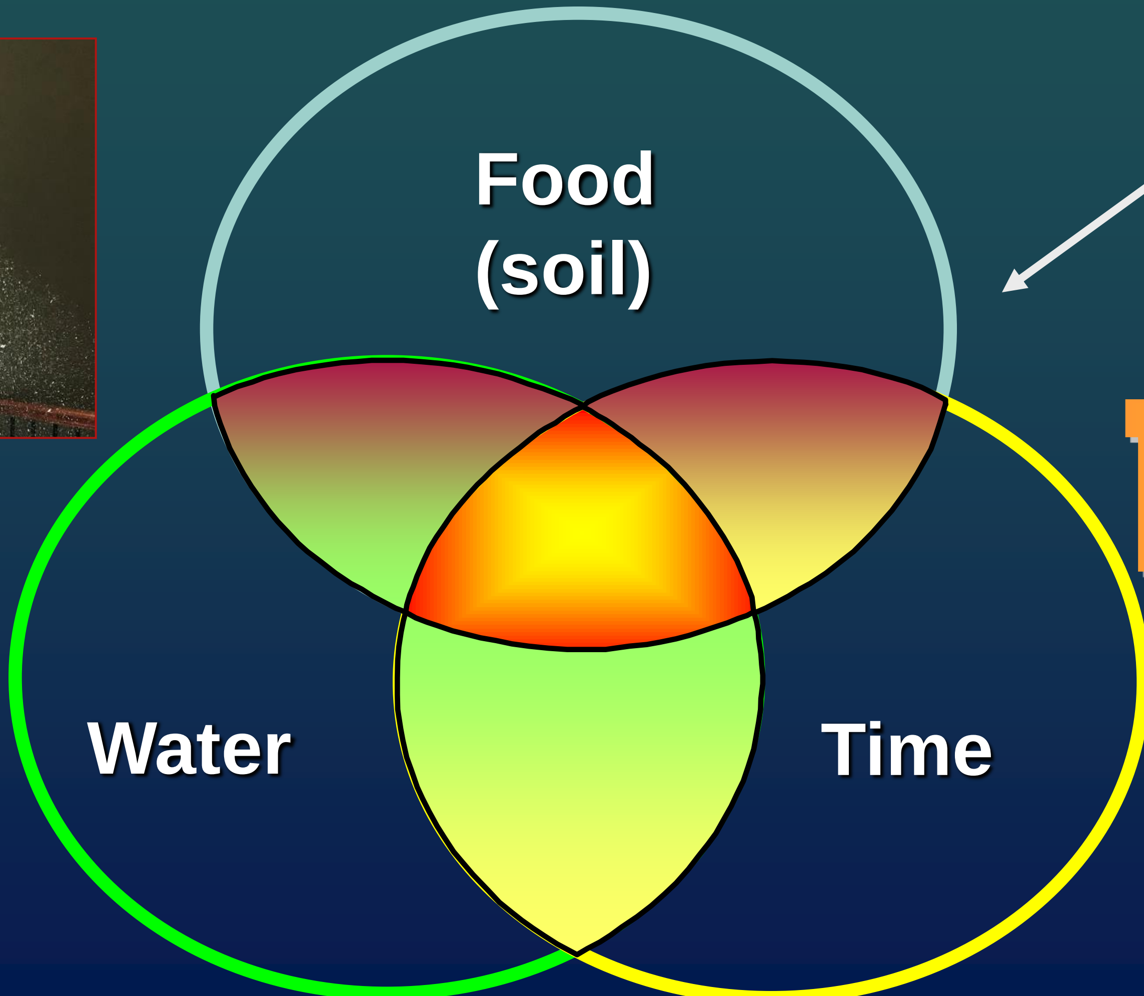
So, Where are they?

"Chance favors only the prepared mind"

- Louis Pasteur

KORNACKI MICROBIOLOGY SOLUTIONS, INC.

Microbial Growth Requirements



Organism

Temperature

It is impractical and likely impossible to maintain a sterile food production facility; but we can control microbial growth and niche creation and disruption

Environmental Contamination

The risk of post-process contamination is increased *if*

the product is not biocidally treated in the end-use container

High numbers usually required to inoculate foods at a measurable level

1 x 10^6 cells per ml in a niche; 10,000 #'s of product *~2 cells per 10 gram product*

2. Eliminate the conditions that favor their development (Some "Do's" and "Don'ts")

Microbial Growth Niches and Biofilm Creation and Disruption

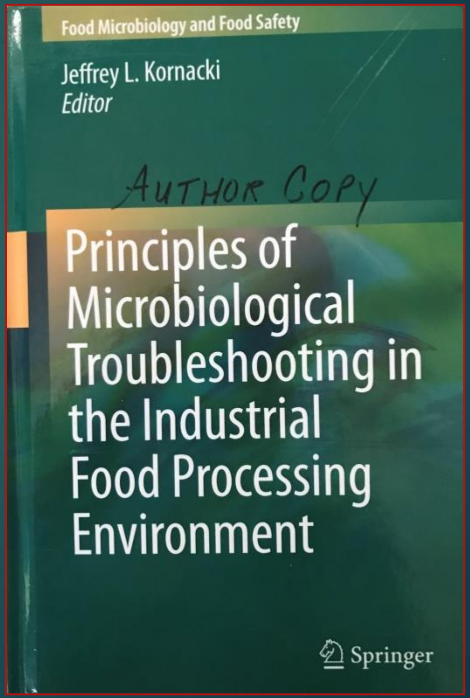
(Overlapping Categories)

Operating practices (e.g., misapplied sanitation)

Maintenance / repair practices

Design / fabrication of factory / equipment

https://www.springer.com/us/book/9781441955173



Misapplied Sanitation of a Rotary Valve Used in a Low a_w Bagging Operation

Design of Facility and Location of Cleaning Implements

Kornacki Microbiology Solutions, Inc.

Importance of Preventative Maintenance Programs

"Have an *equipment maintenance record and monitoring program* to check for broken, pitted, rusty, peeling or dirty, equipment that need replacing, repair, cleaning, etc."

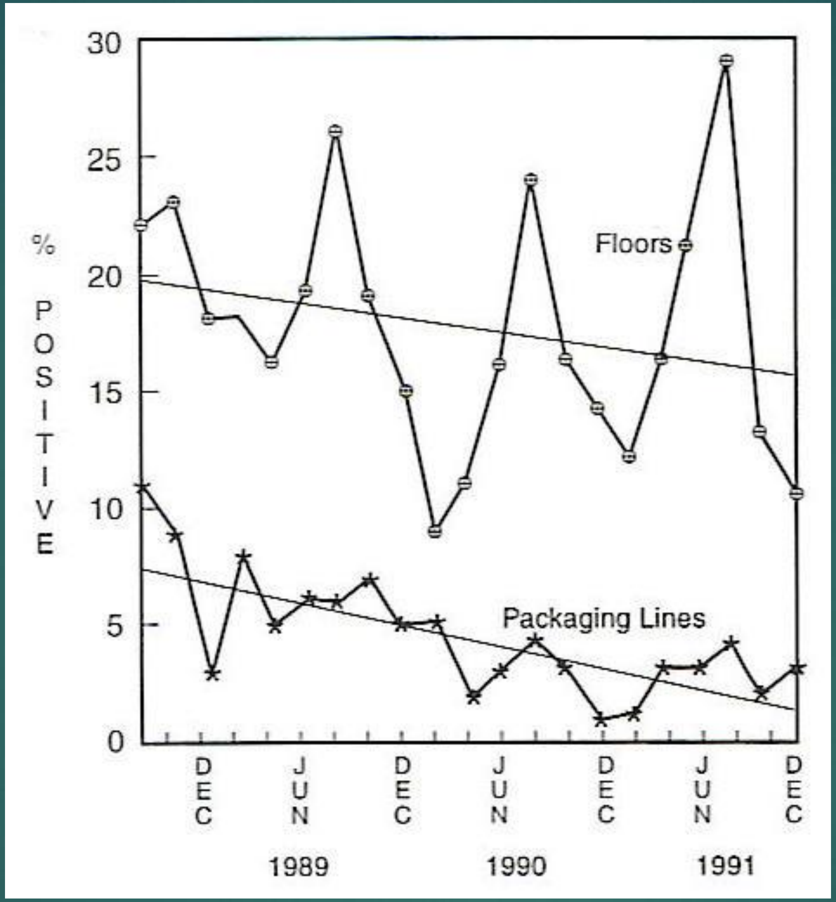
Design of Plant: Flat Roofs and Water Leaks

“Immediately fix leaky roofs, broken and cracked equipment, floors, doors, windows, etc.”

Sloped roofs

much better

Correlation of % *Listeria spp.* Isolated from Packaging Lines and Floors to RTE Meat



Lead to in-plant risk assessment concept

Tompkin, R.B., L.N. Christiansen, A.B. Shaparis, R.L. Baker, and J.M. Schroeder. 1992. Control *of Listeria monocytogenes* in processed meats. Food Australia 44:370-376

Kornacki, J. L. and J. B. Gurtler. 2007. Incidence and control of Listeria in food processing facilities, Chapter 17. In, E. T. Ryser and E. H. Marth (eds.), *Listeria, listeriosis and food safety*, 3rd ed. CRC Press, Taylor & Francis Group, Boca Raton, FL. Pp. 681-766.(see page 729).

Correlations of % Environmental to % Finished Product Contamination

Smoked fish plant: Correlation of environmental *L. monocytogenes* to finished product ($p<0.0001$)

Thimothe et al. 2004. Tracking of *Listeria monocytogenes* in smoked fish processing plants. J. Food Prot. 67(2):328-341.

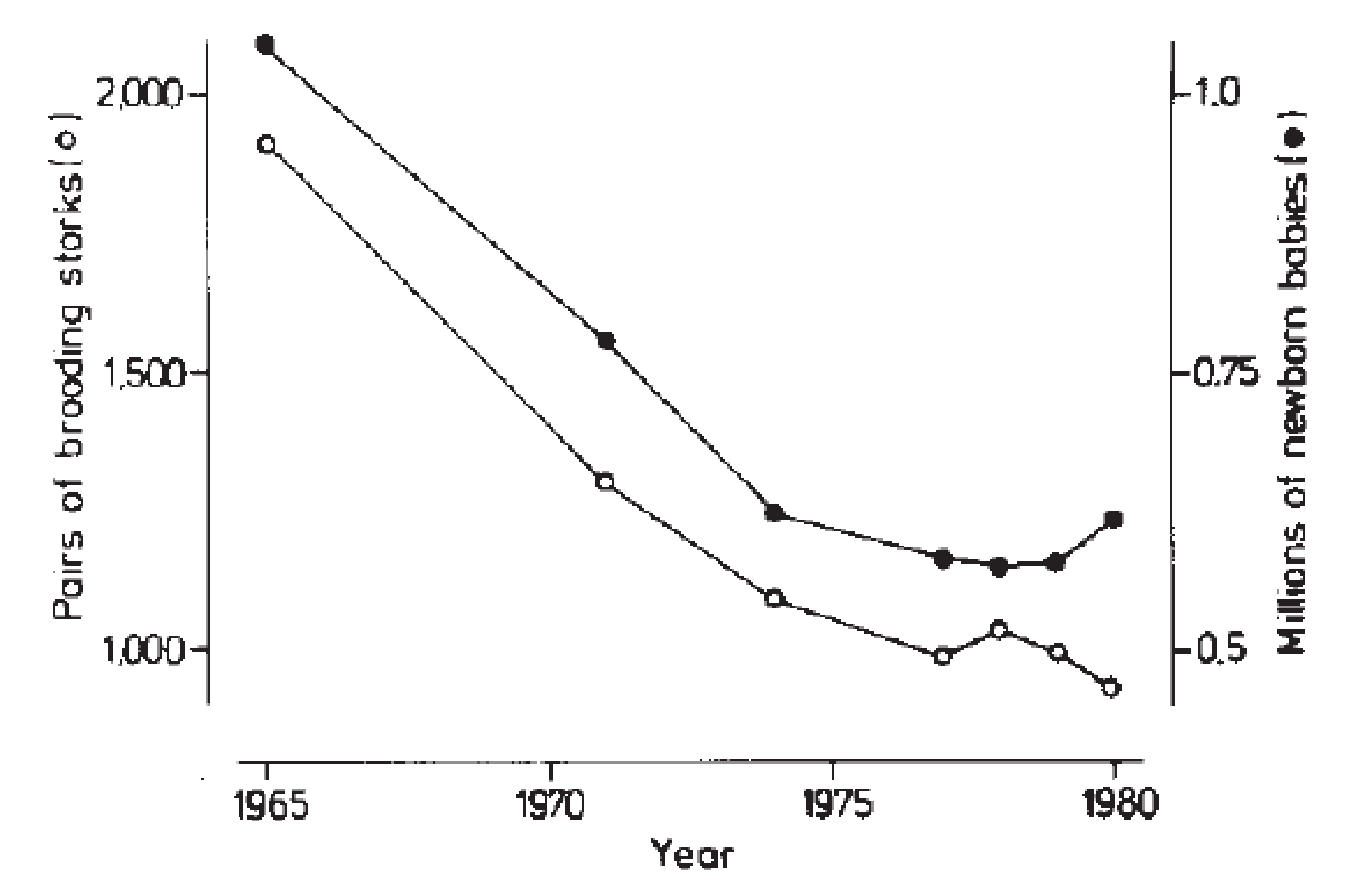
Variables Affecting Likely Contamination From the Processing Environment

"The *probability* of product contamination from the environment is dependent upon a number of variables…"

1. *Proximity of microbial growth niches to the product stream (e.g., processing equipment, zone 1 and 2)*
2. *No. of niches in the food production facility*
3. *Spatial relationships of niches and product stream*
4. *Microbial population in niches*
5. *Degree of niche disruption during operations*
6. *Exposure of the product stream to the environment (e.g., processing equipment zones 1 and 2)*

Gabis, D. A. and R. E. Faust. 1988. Controlling microbial growth in the food-processing environment. Food Technol. Dec. pp. 81-82.; 89.

Exceptions to the Rule: Correlation Vs Causation



Sies, H. 1988. A new parameter for sex education
Nature 332, 495-495

EMP Can be a Profit Center

Sanitation

How and Why Environmental Monitoring Programs Add to the Bottom Line

February 26, 2021

Jeffrey L. Kornacki Ph.D.

Over three decades of active consultation with the food industry, largely on microbiological matters related to investigation, risk assessment, and control, I have seen companies in both the best of times and the worst of times. During hundreds of visits to many food

Kornacki, J.L. 2021. How and Why Environmental Monitoring Programs Add to the Bottom Line. Food Safety Magazine. February/March issue.

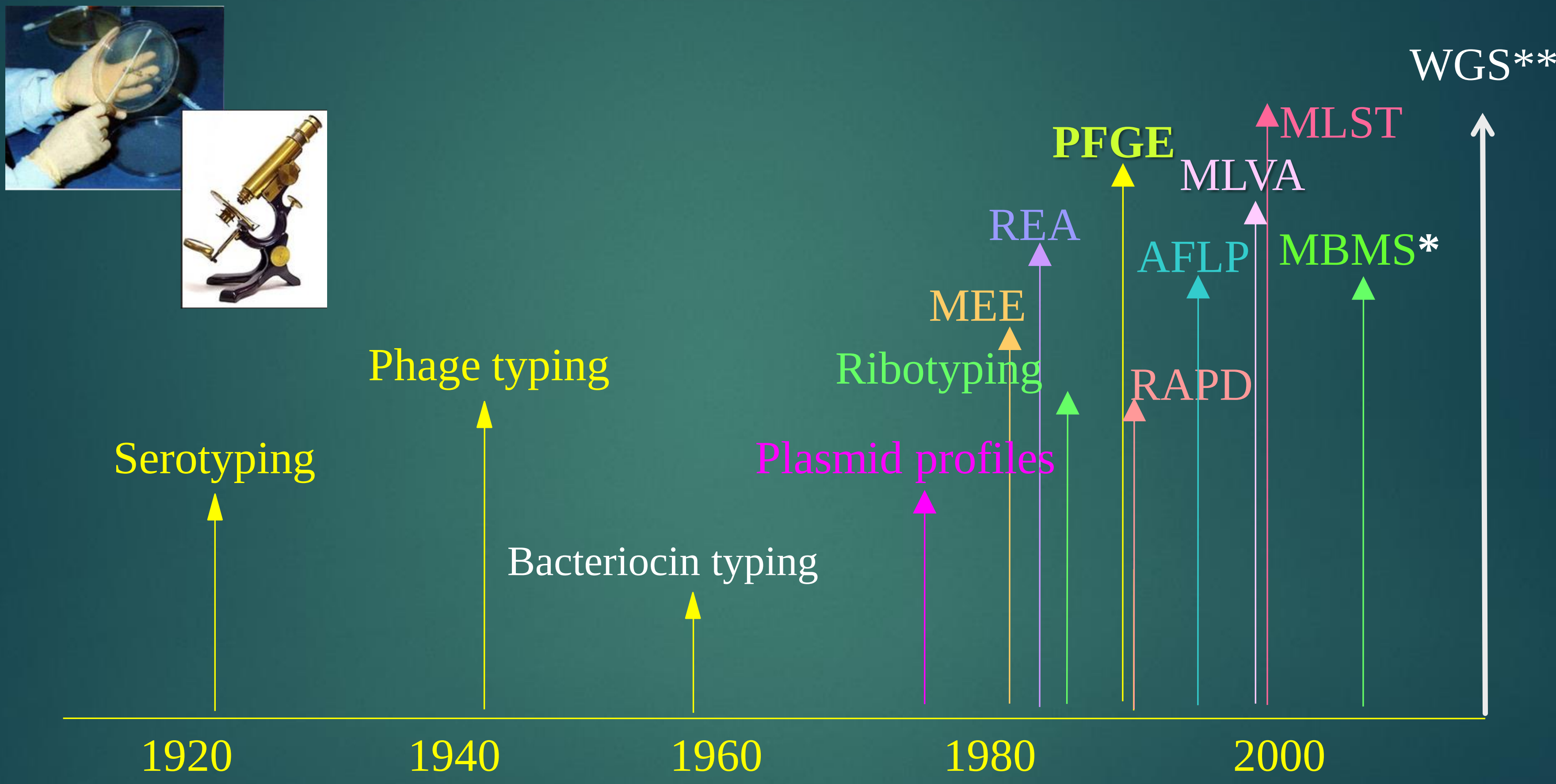
Tracking Strains

- A variety of molecular techniques can be applied

- Common approaches- Genetic (e.g., Rep PCR[1], Ribotyping[1], PFGE[1], MLST, WGS), and phenotypic (surface chemistry; serotyping, FTIR)

[1]**Moorman, M., P. Pruett, M. Weidman. 2010. Value and methods for molecular subtyping of bacteria, Chapter 10. *In*, J. L. Kornacki (ed.), *Principles of Microbiological Troubleshooting in the Industrial Food Processing Environment*, Springer, New York. Pp. 157-174.**

Trends of “Fingerprinting” for Bacteria

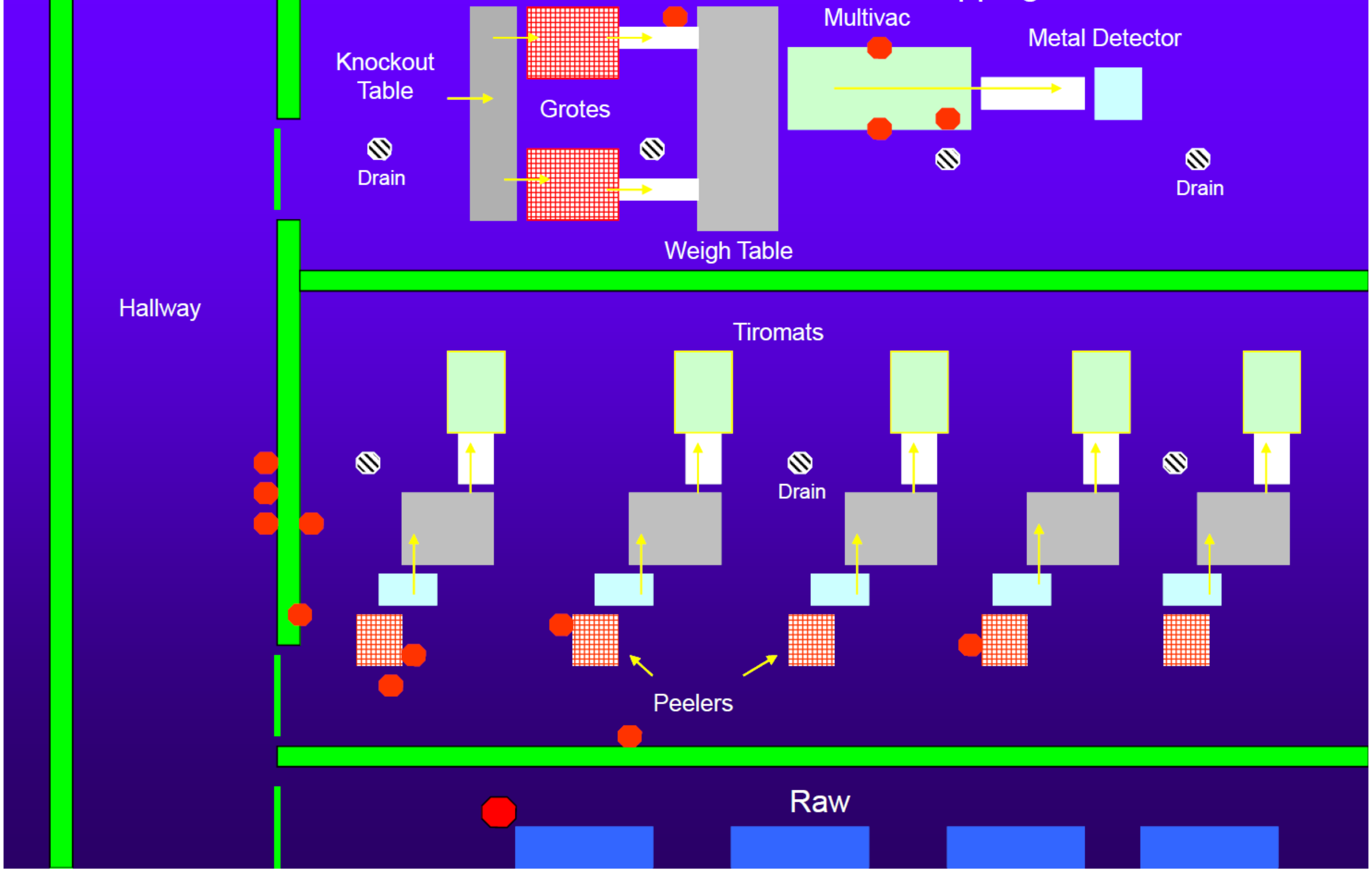


* Microarray-based multi-target sequencing

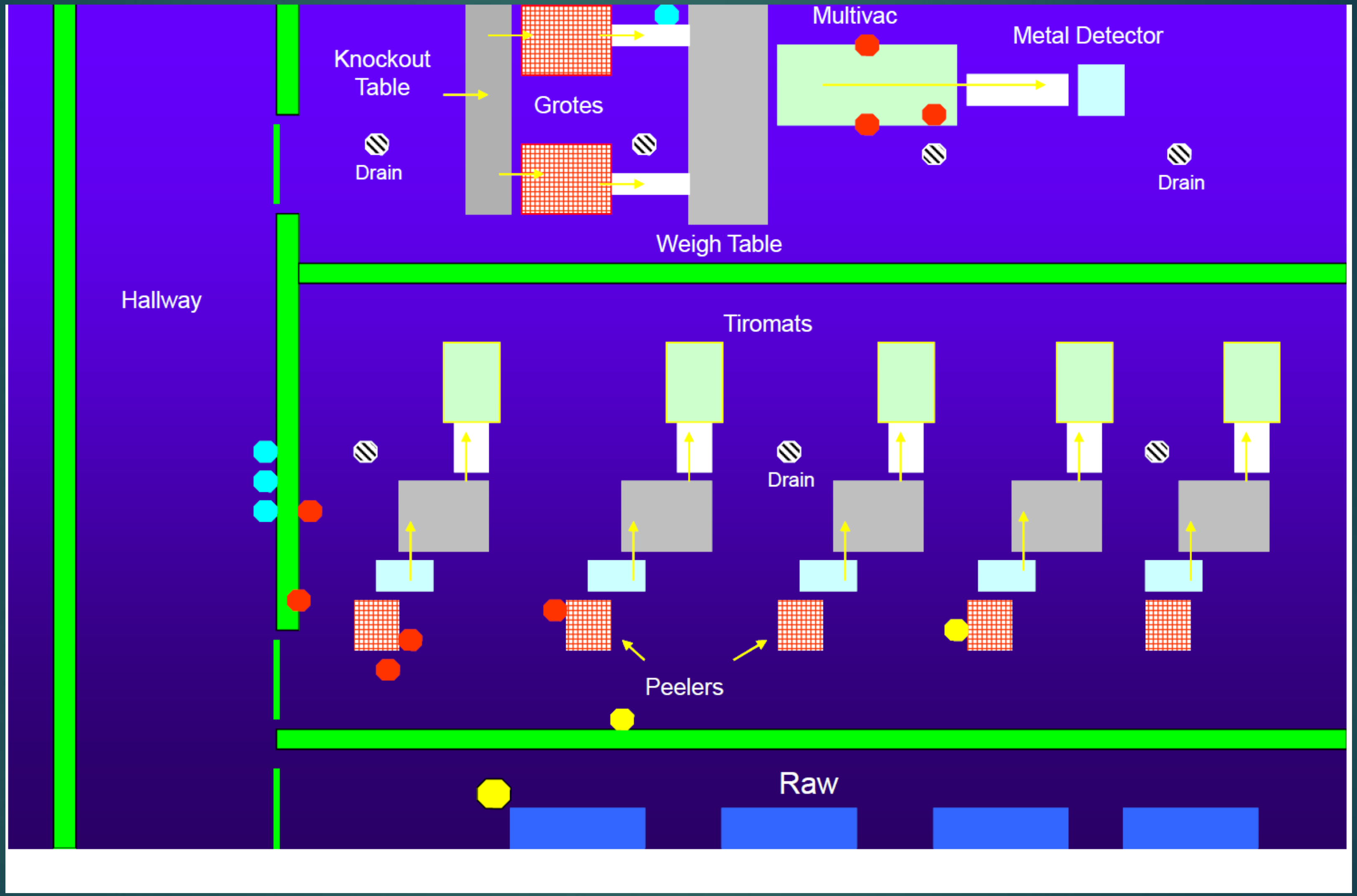
** Whole Genome Sequencing

Slide Courtesy of Art Liang, CDC

Current Practices for Environmental Mapping



Environmental Map with Comparison of Indicator Isolates



WGS VS. Other Approaches: An Industrial Perspective Or To Test or Not To Test?

It Depends

Parking Tickets Vs. Parking Permits

PFGE and WGS Called a "Fingerprint"

- "PulseNet compares the DNA *fingerprints* of bacteria from patients to find clusters of disease that might represent unrecognized outbreaks." CDC in reference to PFGE
- https://www.cdc.gov/pulsenet/pathogens/pfge.html
- "…PulseNet has transitioned from PFGE to Whole Genome Sequencing (WGS)".

https://www.cdc.gov/pulsenet/pathogens/pfge.html

What is a Fingerprint?

Noun- "An Impression or mark made on a surface by *a person's* fingertip, able to be used for identifying from the unique pattern of whorls and lines on the fingertips. - Oxford Dictionaries

"The impression or mark left by the underside of the tips of the fingers or thumbs. The impression is formed by a pattern of ridges on the skin surface. This pattern is unique for each individual and therefore can serve as a means of identification.

http://www.dictionary.com/browse/fingerprint

Bacteria

- Exist as populations not as individuals
- They can be cloned, they are not as complex as humans, but they can mutate

(3 million vs 3 billion base pairs)

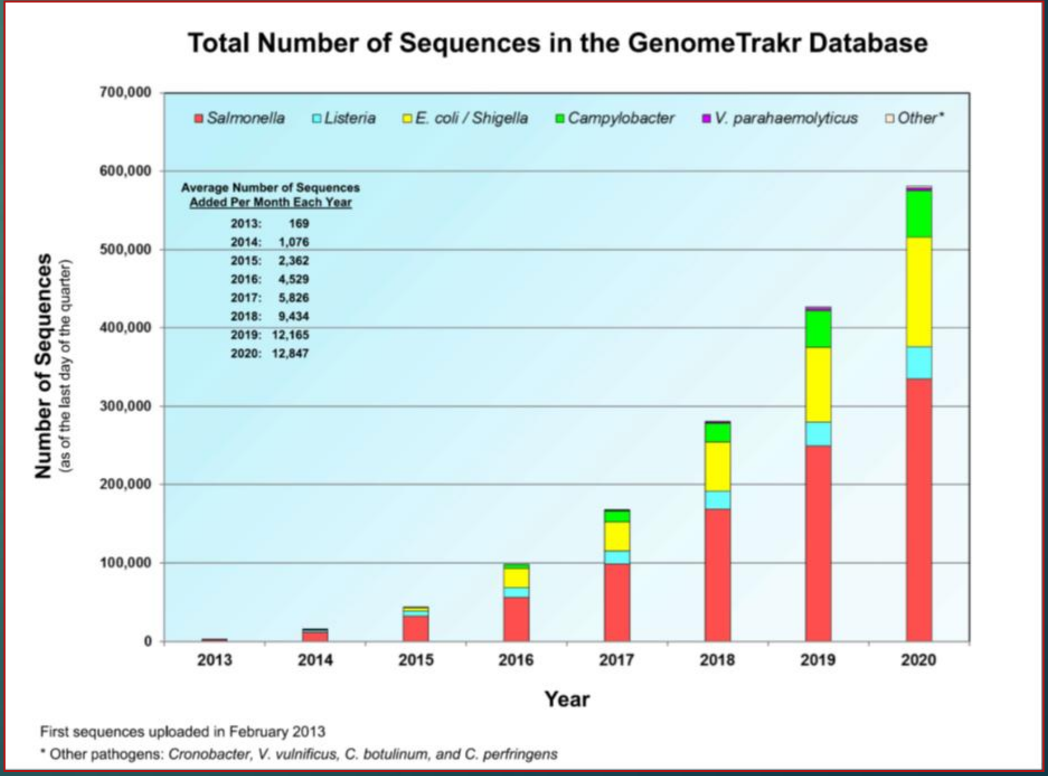
If You Don’t Have a Parking Ticket

WGS -Not a good idea for routine environmental samples in any of your Zones

Remember the WGS Draft Sequences

FDA records access

(Plausible scenario or not?)



Correlation Vs Causation: Plausible Scenario and Perceptions

"*Nothing So Complicated as Perception*" – Anij to Jean Luc Picard - Star Trek Insurrection

- **Can two strains that are unrelated geographically be the same strain?**

The Evil Identical Twin Who Moved to England and Robbed Banks

- Same clone
- Same WGS pattern
- No causal relationship with the good twin living in America
- But what if that twin visited England?
- Might he be arrested?

Hypothetical Cases

Case 1: No Parking Ticket

Environmental WGS pattern “A” in manufacturing environment

Popular band coast-to-coast and widely consumed

WGS pattern “A” in Clinical Samples: 5 years ago

Plausible scenario? A slippery slope

Chances of a recall/regulatory action?

Case 2: No Parking Ticket

History of environmental samples with WGS pattern "B"

(Persistent strain)

Also, product with WGS pattern "B" which company reprocessed (e.g., repasteurized, re-treated, etc.): No positive product released

No illness

FDA visit and records access

Recall/regulatory action?

Root cause?

Case 3: No Parking Ticket

Clinical cases of WGS pattern “C”

Product with WGS pattern “C” which was reprocessed and sold

Popular brand

Recall/regulatory action?

Root cause?

Case 4: Parking Ticket

Product tests positive for WGS pattern "D" AND you have filed an RFR and are in a recall

Do a Root Cause Investigation, best to do this under Attorney Client Privilege (Levitt[1])

Test post-lethality ***ingredients***: Do WGS

But do it in a *Statistically Robust* manner

Test the Environment: Do WGS

Why?

Food Safety News

A seat at the enforcement table: 'I wish I would have acted sooner'

By Guest Contributor on March 5, 2021

CONTRIBUTED OPINION

By Joseph A. Levitt

[1]**Levitt, J. 2021. A seat at the enforcement table, "I wish I had acted sooner." Food Safety News. March 5.**

Why?

"In Order for the Government to Feel Comfortable to Let You Operate they Need To Know that the Root Cause was *Found and Eliminated.*"

Environmental Root Causes

Three Types

1. How did the microorganism get into the product? (Usually a Zone 1-2 investigation)

2. How did the microorganism get into the equipment (if that was the source; usually a Zone 3-4 investigation)

3. How did the microorganism get into the building or the processing area (traffic, drains, HVAC, Air flow)

Root Cause 1: Post-Process Preventive Control added Ingredients

Test Number Needed to Detect One or More Positives per Lot

Percent positives	Number of analytical units to be tested (n)		
% Positive	90 % confidence	95 % confidence	99 % confidence
100	3	4	4
10	23	30	46
1	230	299	461
0.1	2,303	2,996	4,605
0.01	23,026	29,963	46,052

Adapted:Compendium of Methods for the Microbiological Examination of Foods 3rd ed.

Table 2-2. Fraction Positive Samples When the Probability Is That All *n* Samples Are Negative

	Fraction Positive Samples (P)		
	Probability (Pr)		
Number of Analytical Units (*n*)	0.10	0.05	0.01
3	0.77†	1.00	1.50
5	0.46	0.60	0.92
10	0.23	0.30	0.46
15	0.15	0.20	0.31
20	0.12	0.15	0.23
25	0.092	0.12	0.18
30	0.077	0.10	0.15
35	0.066	0.086	0.13
40	0.058	0.075	0.12
45	0.051	0.067	0.10
50	0.046	0.060	0.092
100	0.023	0.030	0.046
200	0.012	0.015	0.023
400	0.0058	0.0075	0.012
500	0.0046	0.0060	0.0092
1000	0.0023	0.0030	0.0046

Taylor, et al. 2015. Sampling plans, sample collection, shipment, and preparation for analysis, Chapter 2. In, Y. Salfinger and M. L. Tortorello (Eds.), *Compendium of Methods for the Microbiological Examination of Foods*. APHA, Washington, D.C. Pp. 13-25.

Environmental Post Process- Preventive Control Sites

All Zones

RC 1: Extensive equipment break down and swabbing: Avoid paradigms that prevent problem solving

RC 2: Extensive Zone 3-4 sampling

RC 3: Traffic into the building (birds, trucks and loading docks), roof leaks, backed up drains

Alternatives for Those Without a Parking Ticket

- HQA/HTEB (*Listeria*-like and *Salmonella*-like organisms, respectively)
- And REP PCR or Riboprinting with Unique Restriction Endonucleases or phenotypic Biotyping (e.g., FTIR approach).

Tracking Strains: Parking Tickets Vs. Parking Permits

- *Listeria* and *Listeria*-like organisms with Ribotyping, REP PCR, Biotyping
- HTEB assay with Rep PCR or Biotyping

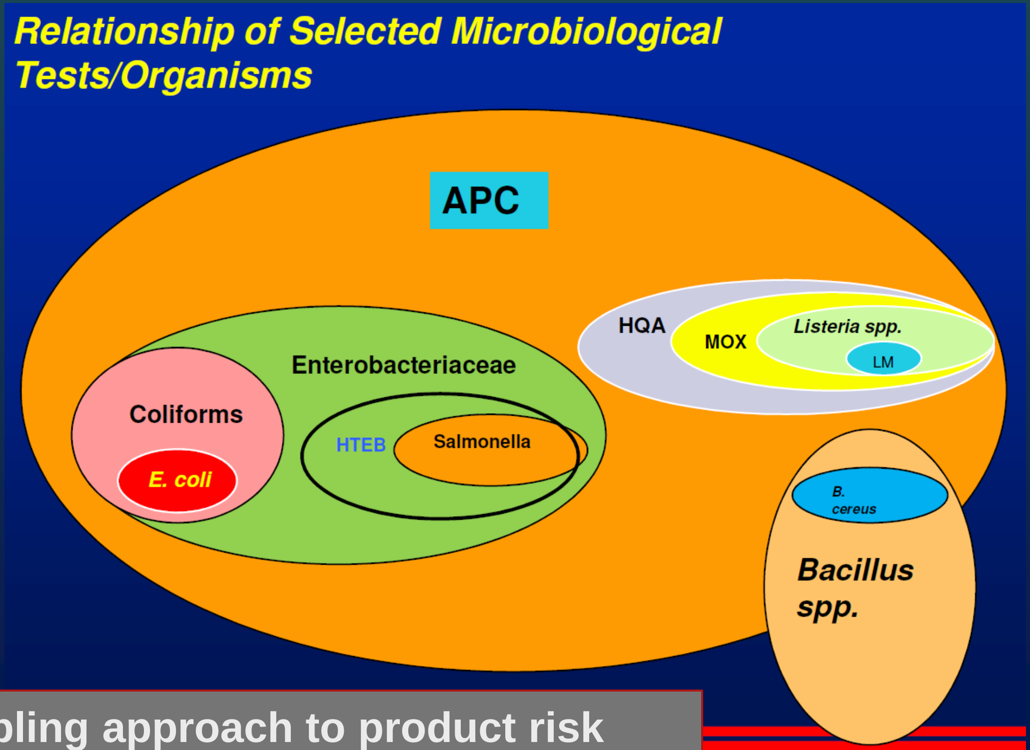
Multiple subtypes suggest multiple sources and can inform the investigation


TESTING

By Jeffrey L. Kornacki, Ph.D.

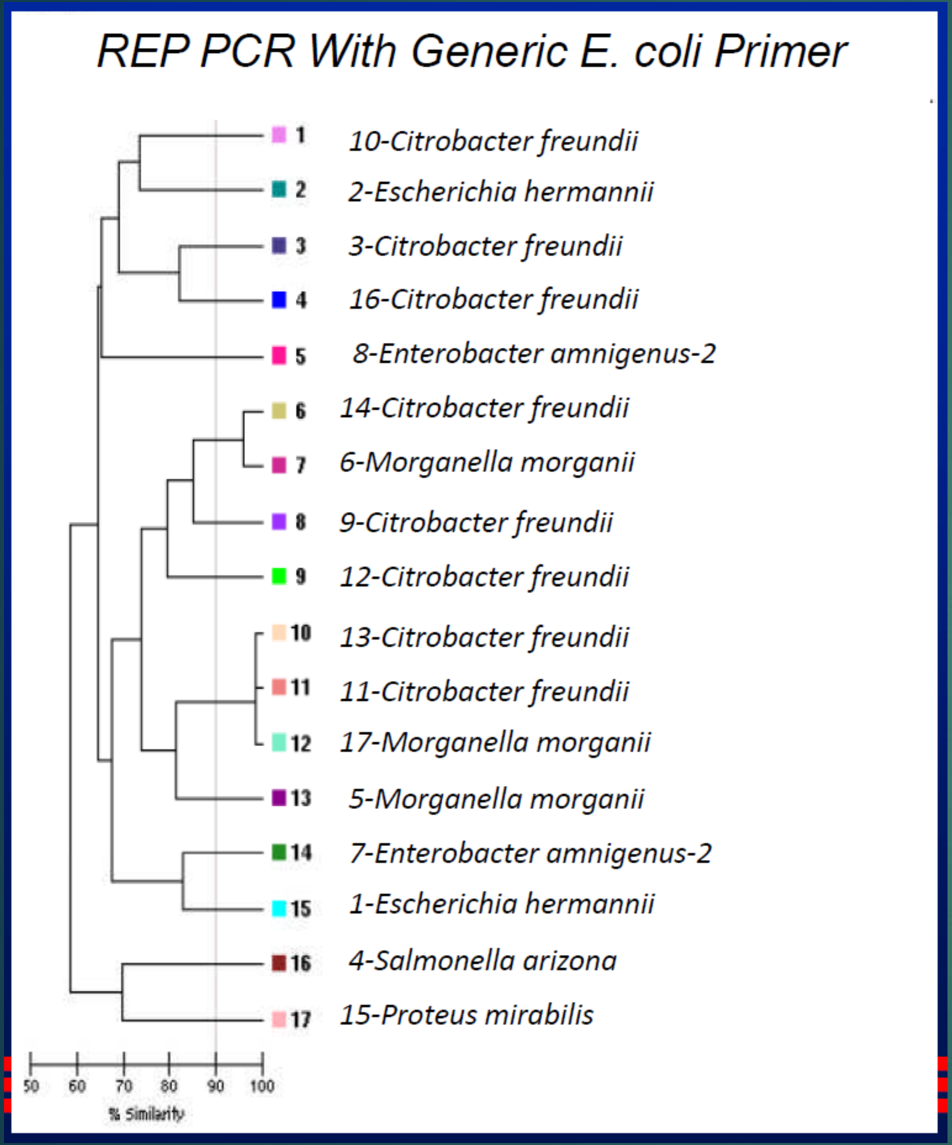
An Environmental Sampling Approach to Product Risk Assessment

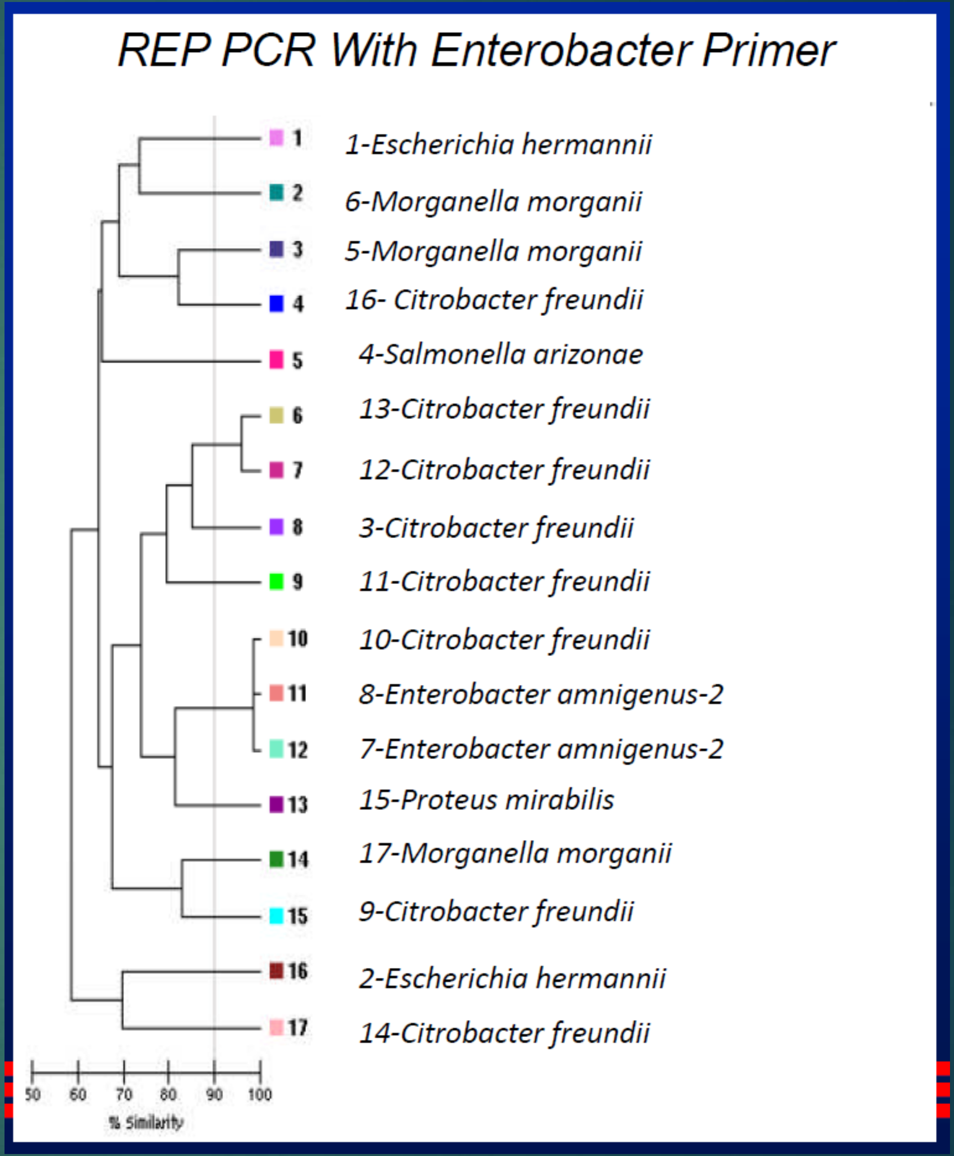
Objective, product-line-specific testing imperative for environmental risk analysis



Kornacki, J. L. 2014. An environmental sampling approach to product risk assessment. Food Safety Magazine. February/March issue.

HTEB Subtypes in a Limited Investigation of a Low a_w Food Processing Facility





Summary: Selected Capabilities of Microorganisms

"Bacteria are smarter than we are because they do not have a brain to worry about"

– R. Behling

Their astounding capabilities makes them difficult to control

Summary

Microbes are highly adaptable and successful creatures

Environmental contamination is likely to be the most significant source of finished product contamination

Persistent strains in processing plant environments may result from biofilms rendering them more resistant to sanitizers and thermal inactivation

Investigations of product contamination should be done with robust (not routine) statistical sampling of ingredients that are not subjected to a lethal treatment.

Investigations should include observations and appropriate sampling in areas related to operating practices, maintenance & repair practices, and appropriate sanitary design of the facility and equipment

Persistent strains can be tracked with various molecular subtyping approaches

Consider all the consequences to any molecular subtyping approach you choose

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Low Water Activity Food Safety Series

Please join us for these webinars:

Part 2: Microbiological Safety of Nuts and Products	April 15, 11:00 AM (EDT)
Part 3: Microbiological Safety of Dried Spices	May 12, 11:00 AM (EDT)
Part 4: Grain Based Foods and Ingredients	June 9, 11:00 AM (EDT)

This webinar is being recorded and will be available for access by **IAFP members** at www.foodprotection.org within one week.

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