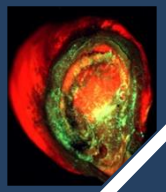
Food Safety and Intervention Technologies-Brendan A. Niemira, PhD., Research Leader

The Role of Genotype in the Development and Validation of Growth Models and Intervention Technologies for Pathogenic Non-Shiga Toxigenic *Escherichia coli* Found in Foods *LS: Chris Sommers*

Development of Alternative Intervention Technologies for Fresh or Minimally Processed Foods

LS: Brendan A. Niemira



Development of Detection and Intervention Technologies for Bacterial and Viral Pathogens Affecting Shellfish

LS: Gary Richards

Development and Validation of Innovative Food Processing Interventions

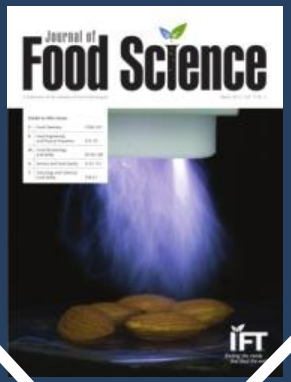
LS: David Geveke

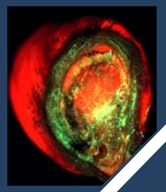
Bacterial Pathogens in Regulated Foods and Processing Technologies for Their Elimination *LS: John Luchansky*

Food Safety and Intervention Technologies-Brendan A. Niemira, PhD., Research Leader

Development of Alternative Intervention Technologies for Fresh or Minimally Processed Foods

LS: Brendan A. Niemira





The Role of Genotype in the Development and Validation of Growth Models and Intervention Technologies for Pathogenic Non-Shiga Toxigenic *Escherichia coli* Found in Foods *LS: Chris Sommers*

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Development and Validation of Innovative Food Processing Interventions

LS: David Geveke

Bacterial Pathogens in Regulated Foods and Processing Technologies for Their Elimination *LS: John Luchansky*

Efficacy of a New Fresh Produce Wash, *First Step+ 10*, at Inactivating Foodborne Pathogens in Rinse Water and on Cut Apples, Cherry Tomatoes, Cantaloupe Rind, and Spinach

JOSHUA GURTLER[†], XIAOLING DONG[‡], STEPHEN SANTOS[‡], RENSUN LEE[‡] AND REBECCA BAILEY[†]

[†]Food Safety Intervention Technologies Research Unit • USDA • Agricultural Research Service • Eastern Regional Research Center • Wyndmoor, PA

[‡]Mantrose-Haeuser Co., Inc. • NatureSeal, Inc.
Research & Development • Lincoln, RI

USDA

ERRC

EASTERN REGIONAL RESEARCH CENTER

NatureSeal

A division of Mantrose-Haeuser Co., Inc.

Cross Contamination in Flume and Dump Tank Water Is Very Problematic

Commercial antimicrobials aren't always optimal in the presence of organic matter

Established a USDA, ARS CRADA to Create an Advanced Organic Antimicrobial Produce Wash

NatureSeal®

A division of Mantrose-Haeuser Co., Inc.

First Step+ 10 Produce Wash (1)

COMPOSITION

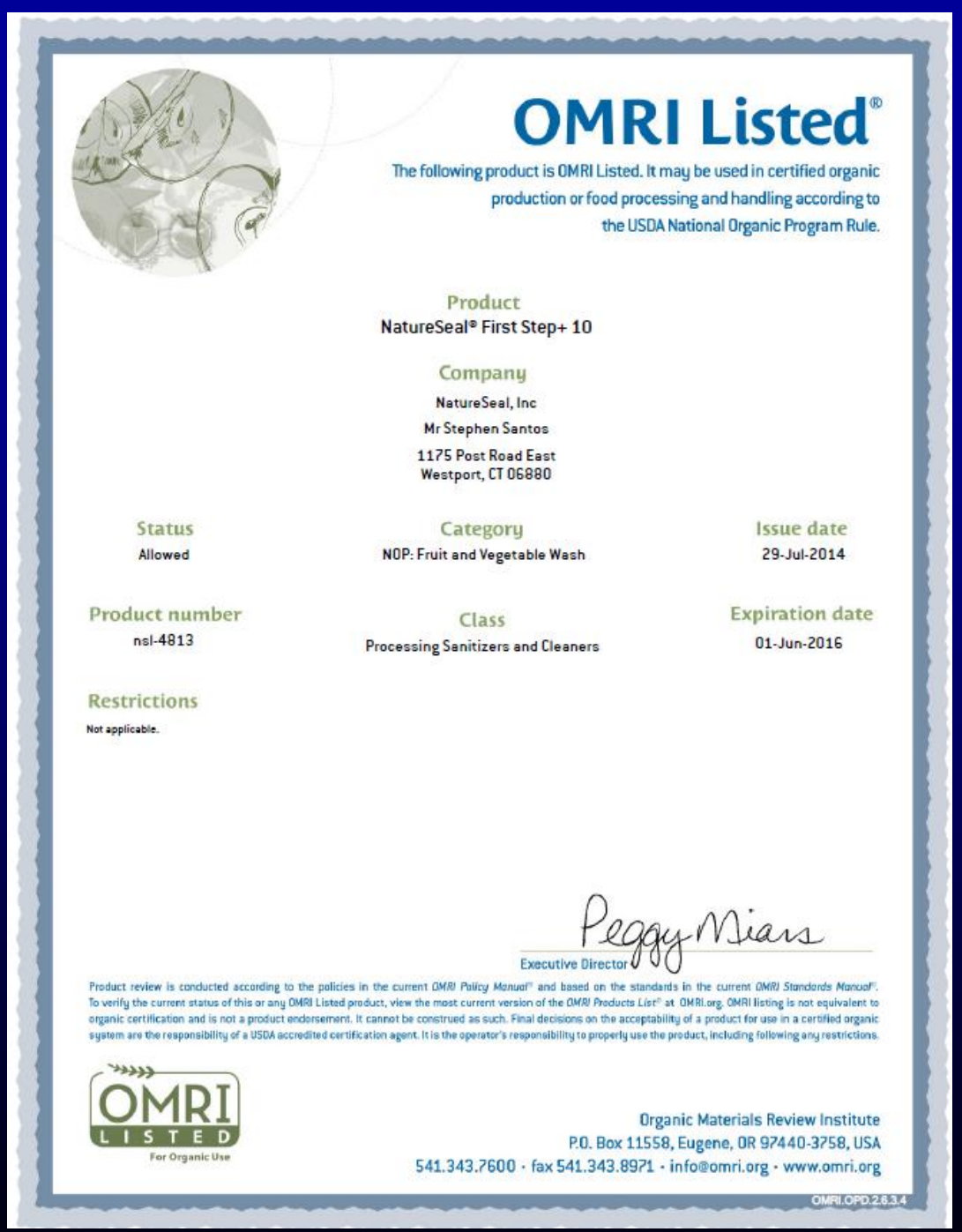
Made up of a combination of one or more fruit acids + lactic acid + H_2O_2, yielding a solution of mixed Peracids (Perorganic Acids)

First Step+ 10 Produce Wash (2)

VALIDATION STUDIES

- Using the British Standard EN 1276 method, ***First Step+ 10*** reduced pathogens *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* by >99.999%.
- Received a non objection letter from Health Canada for use as an antimicrobial produce wash.
- Received FDA FCN approval.
- *NatureSeal* has been working with processors in conducting commercial trials for about 2 years.

Was Issued OMRI, USDA Certified Organic Listing



OMRI Listed®

The following product is OMRI Listed. It may be used in certified organic production or food processing and handling according to the USDA National Organic Program Rule.

Product
NatureSeal® First Step+ 10

Company
NatureSeal, Inc
Mr Stephen Santos
1175 Post Road East
Westport, CT 06880

Status	Category	Issue date
Allowed	NOP: Fruit and Vegetable Wash	29-Jul-2014
Product number	**Class**	**Expiration date**
nsl-4813	Processing Sanitizers and Cleaners	01-Jun-2016

Restrictions
Not applicable.

Peggy Miars
Executive Director

Product review is conducted according to the policies in the current *OMRI Policy Manual*® and based on the standards in the current *OMRI Standards Manual*®. To verify the current status of this or any OMRI Listed product, view the most current version of the *OMRI Products List*® at OMRI.org. OMRI listing is not equivalent to organic certification and is not a product endorsement. It cannot be construed as such. Final decisions on the acceptability of a product for use in a certified organic system are the responsibility of a USDA accredited certification agent. It is the operator's responsibility to properly use the product, including following any restrictions.

OMRI LISTED
For Organic Use

Organic Materials Review Institute
P.O. Box 11558, Eugene, OR 97440-3758, USA
541.343.7600 · fax 541.343.8971 · info@omri.org · www.omri.org

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United States Patent Application Publication

(19) **United States**

(12) **Patent Application Publication**

Dong et al.

(10) **Pub. No.: US 2014/0348945 A1**

(43) **Pub. Date: Nov. 27, 2014**

(54) **ANTIMICROBIAL WASH**

(71) Applicants: **Nature Seal Inc., a Delaware corporation**, Lincoln, RI (US); **The United States of America as Represented by the Secretary of Agriculture**, Washington, DC (US)

(72) Inventors: **Xiaoling Dong**, North Attleboro, MA (US); **Joshua Brandt Gurtler**, Phoenixville, PA (US); **RenSun Lee**, North Grafton, MA (US); **Stephen A. Santos**, Cumberland, RI (US)

(73) Assignees: **Nature Seal Inc., a Delaware corporation**, Lincoln, RI (US); **The United States of America as Represented by the Secretary of Agriculture**, Washington, DC (US)

(21) Appl. No.: **14/284,453**

(22) Filed: **May 22, 2014**

Related U.S. Application Data

(60) Provisional application No. 61/826,775, filed on May 23, 2013.

Publication Classification

(51) **Int. Cl.**

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A01N 37/02	(2006.01)
A01N 37/04	(2006.01)
A23B 7/157	(2006.01)
A01N 59/00	(2006.01)

(52) **U.S. Cl.**

CPC . ***A23B 7/10*** (2013.01); ***A23B 7/157*** (2013.01); ***A01N 59/00*** (2013.01); ***A01N 37/04*** (2013.01); ***A01N 37/02*** (2013.01)

USPC **424/616**

(57) **ABSTRACT**

A new antimicrobial wash for treating fresh fruits and vegetables to reduce microorganisms, especially human pathogens, comprises an aqueous solution of hydrogen peroxide and one or more fruit acids.

Inactivation of *E. coli* O157:H7 in Crop Soil by Amending with Fast and Slow Pyrolysis-Generated Biochars

JOSHUA GURTLER,
AKWASI BOATENG and DAVID DOUDS

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Wyndmoor, PA

Contamination and Survival of Pathogens in Crop Soil

Improperly composted manure, wild or domestic animals, improper farm laborer hygiene, irrigation or herbicide waters, and run off water from livestock operations

www.mlive.com/midland/index.ssf/2011/08/captured_feral_pig_in_midland_county_tests_positive_for_pseudo-rabies_agriculture_officials_says.html

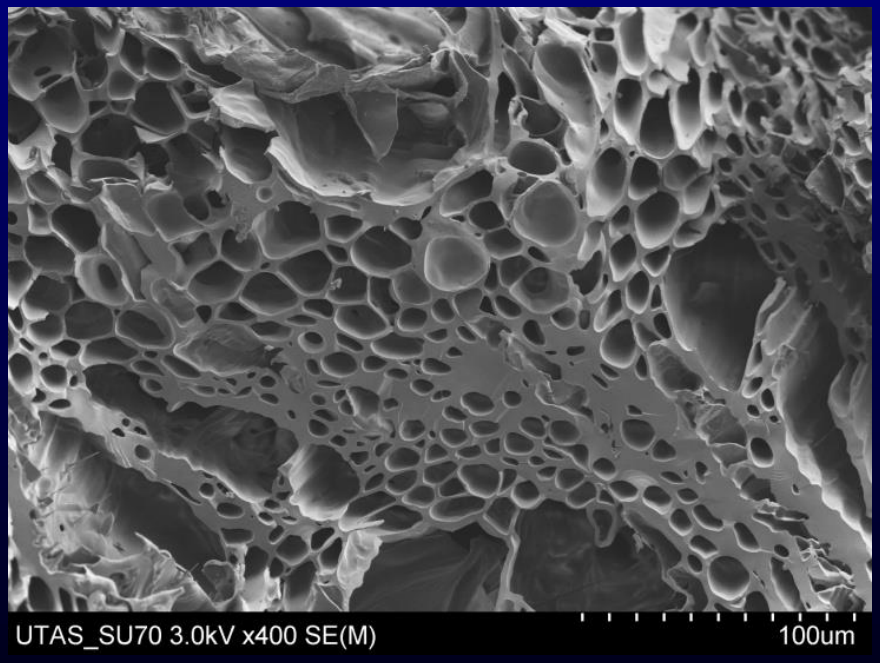
paulfoley.photoshelter.com

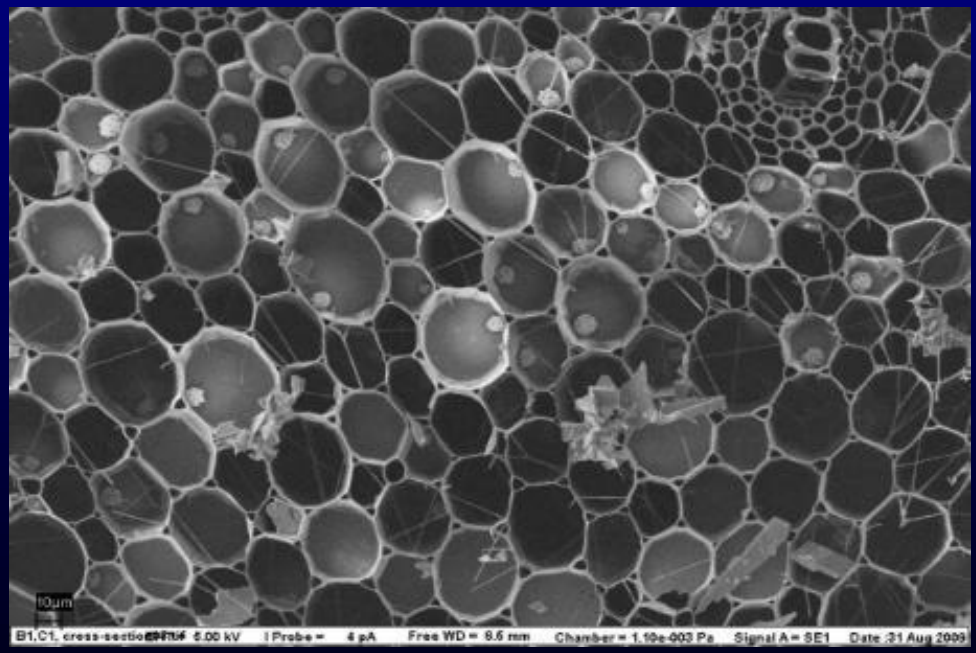
ksre.ksu.edu/conservation/livestock.htm

Fluidized Bed Fast Pyrolysis System

Biochar

- Black carbon generated by pyrolysis resulting in incomplete combustion.
- Very fine, grainy, highly porous material that ranges in size from ca. 200 – 1,000 µm in diameter.
- High concentration of volatiles from short "fast pyrolysis" biomass residence time (< 1 sec)
- Up to 800 m^2/g surface area





FOODBORNE PATHOGENS AND DISEASE
Volume 11, Number 3, 2014

DOI: 10.1089/fpd.2013.1631

Inactivation of *E. coli* O157:H7 in Cultivable Soil by Fast and Slow Pyrolysis-Generated Biochar

Joshua B. Gurtler,[1] Akwasi A. Boateng,[2] Yanxue (Helen) Han,[2] and David D. Douds, Jr.[3]

Abstract

An exploratory study was performed to determine the influence of fast pyrolysis (FP) and slow pyrolysis (SP) biochars on enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) in soil. Soil + EHEC (inoculated at 7 log colony-forming units [CFU]/g of soil) + 1 of 12 types of biochar (10% total weight:weight in soil) was stored at 22°C and sampled for 8 weeks. FP switchgrass and FP horse litter biochars inactivated 2.8 and 2.1 log CFU/g more EHEC than no-biochar soils by day 14. EHEC was undetectable by surface plating at weeks 4 and 5 in standard FP switchgrass, FP oak, and FP switchgrass pellet biochars. Conversely, EHEC populations in no-biochar control samples remained as high as 5.8 and 4.0 log CFU/g at weeks 4 and 5, respectively. Additionally, three more SP hardwood pellet biochars (generated at 500°C for 1 h, or 2 h, or generated at 700°C for 30 min) inactivated greater numbers of EHEC than did the no-biochar control samples during weeks 4 and 5. These results suggest that biochar can inactivate *E. coli* O157:H7 in cultivable soil, which might mitigate risks associated with EHEC contamination on fresh produce.

SY Team	Months	Milestone	Anticipated Product	Progress/ Changes
JG	12	Validate non-pathogenic bacteria as surrogates for pathogenic bacteria in soil survival studies with biochar	Present data at scientific meetings and publish results in peer reviewed journals	
JG	24	Optimize the antimicrobial efficacy of biochar production (time and temperature), using switchgrass as a model	Present data at scientific meetings and publish results in peer reviewed journals	
JG	36	Optimize the antimicrobial efficacy of biochar production (based on biofeedstocks), using the previously optimized time and temperature production protocol	Present data at scientific meetings and publish results in peer reviewed journals	
JG	48	Test efficacy of biochar to inactivate surrogate bacteria in compost	Present data at scientific meetings and publish results in peer reviewed journals	
JG	60	Scale up experiments to field settings to determine the efficacy of the optimized biochar to inactivate surrogate bacteria	Present data at scientific meetings and publish results in peer reviewed journals	

BIOCHAR PRODUCTION

International Biochar Initiative

FEEDSTOCKS

Biochar production processes utilize cellulosic biomass such as wood chips, corn stover, rice and peanut hulls, tree bark, paper mill sludge, animal manure and most urban, agricultural and forestry biomass residues.

Biomass
- manure
- organic wastes
- bioenergy crops (grasses,willows)
- crop residues

(C) 100%

Bio-fuel
- bio-oil
- hydrogen

(C) 50%

Transport
Energy
Coproducts (oil,cosmetics)
Industry

Pyrolysis

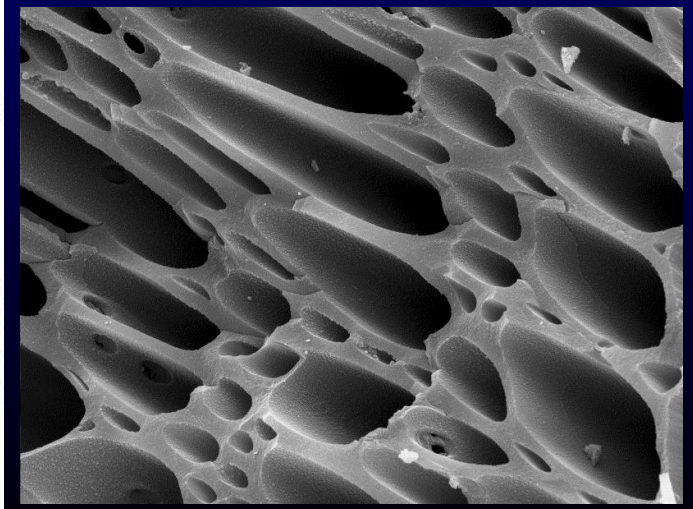
Residual heat

(C) 50%

Returned to soil as **Biochar**

OUTPUTS

Besides biochar, bioenergy is also produced in the form of either synthetic gas (syngas), or bio-oils, which can be used to produce heat, power or combined heat and power.



Mechanism of Antimicrobials for Inactivation Bacteria on Fresh Produce

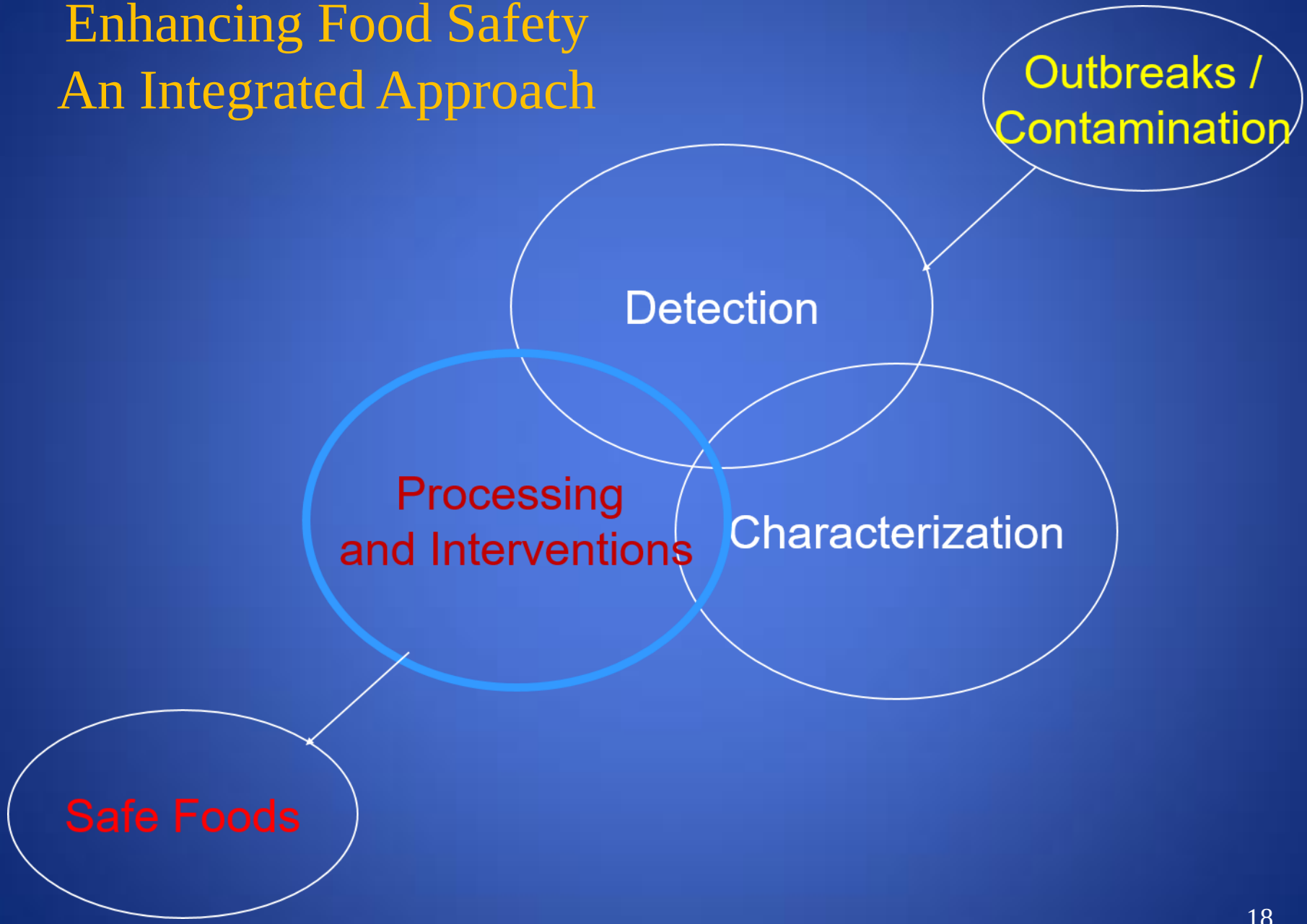
Dike O. Ukuku, Ph.D.

FSIT-ERRC-ARS-USDA

Wyndmoor, PA 19038

Eastern Regional Research Center

Enhancing Food Safety
An Integrated Approach



Antimicrobials

- Antimicrobial agent can be defined as any compound natural and/or synthetic that can slow/inhibit microbial growth and/or kill and reduce microbial populations when used in food processing.
- The agent may induce bacterial injury, leads to recovery or death/inactivation depending on food composition and storage conditions.
- The agents may be classified as chemical and biological, including plant and vegetable associated products found in nature.

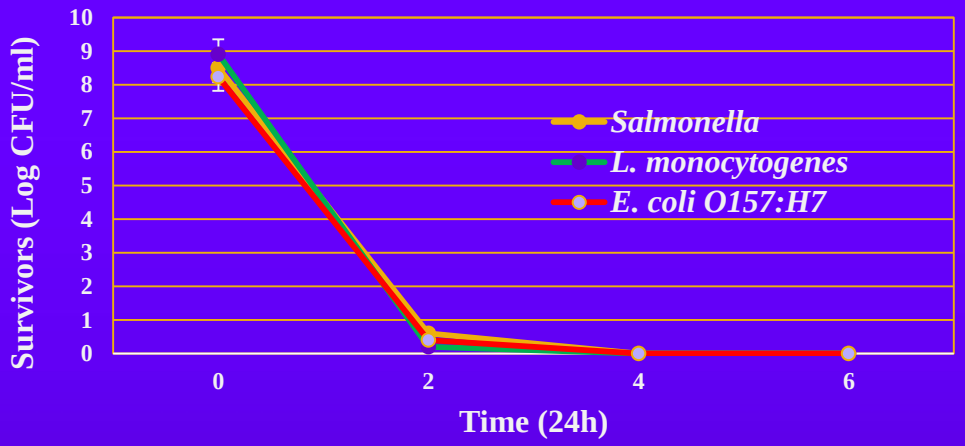
Specific mechanism for most salts used are:

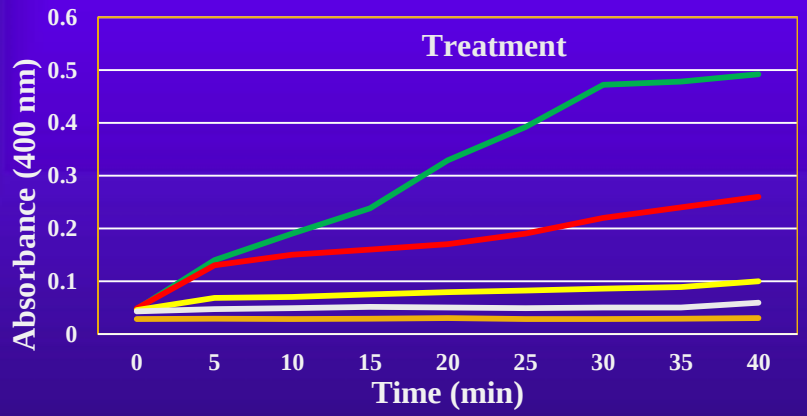
- Direct pH reduction:- Acid dissociation. Un-dissociated form mostly active, penetrates the cell, dissociates and changes internal pH of the organism
- Disrupt substrate transport by altering cell membrane permeability
- Example, can disrupt enzymatic activity that converts pyruvate into products that goes in Krebb cycle
- At ERRC Lab, we developed a new solution (Lovit, licensed, patent pending) with antimicrobial activity.
- Currently working on modifying Lovit to act as antibrowning for fresh-cut apple pieces

Consideration for use

	pH				
Organic Acid	3	4	5	6	7
Acetic	98.5	84.5	34.9	5.1	0.54
Benzoic	93.5	59.3	12.8	1.44	0.14
Citric	53.0	18.9	0.41	0.006	<0.001
Lactic	86.6	39.2	6.05	0.64	0.064
Propylparaben	>99.99	99.99	99.6	99.66	96.72
Propionic	98.5	87.5	41.7	6.67	0.71
Sorbic	97.5	82	30	4.1	0.48

Inactivation of bacteria

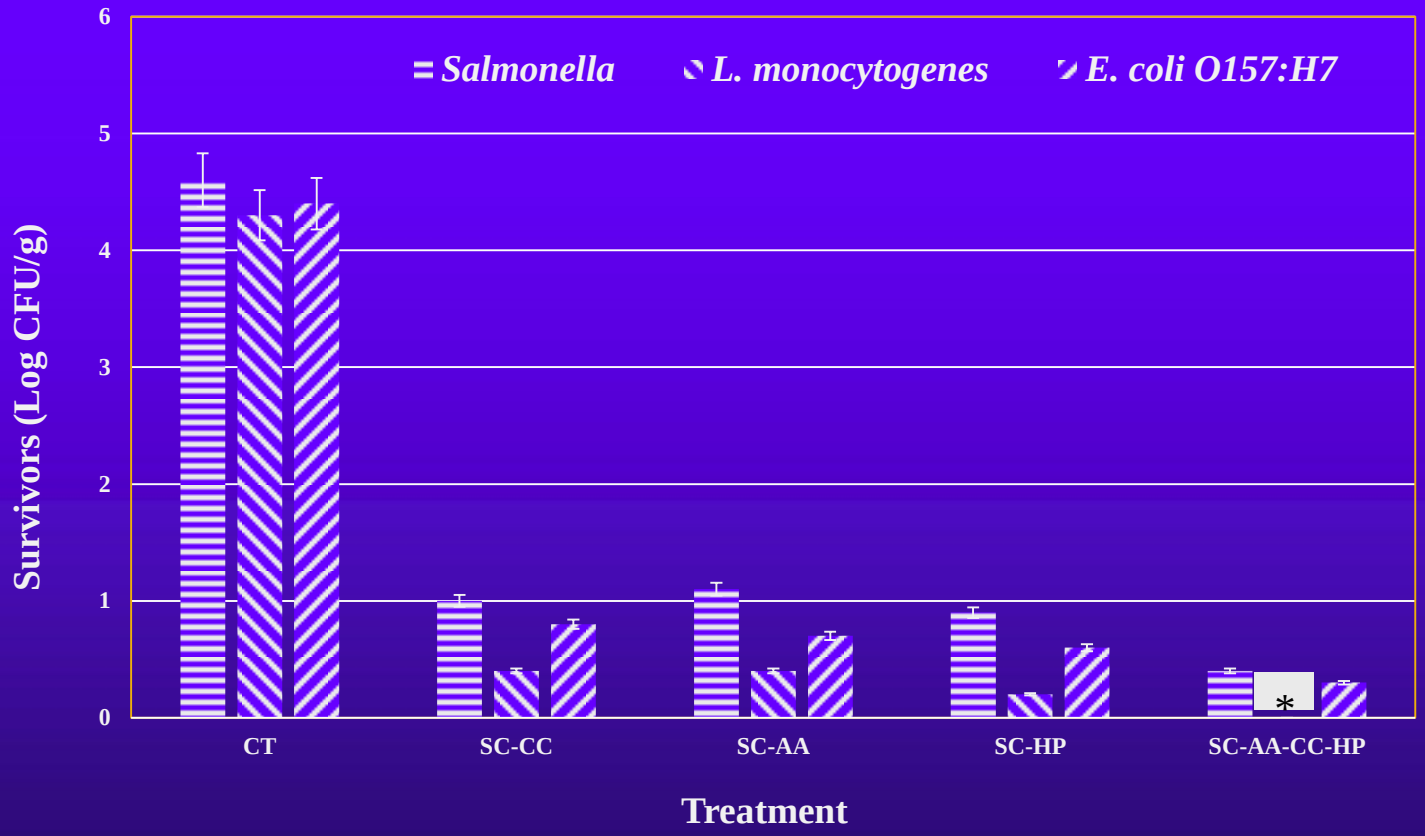




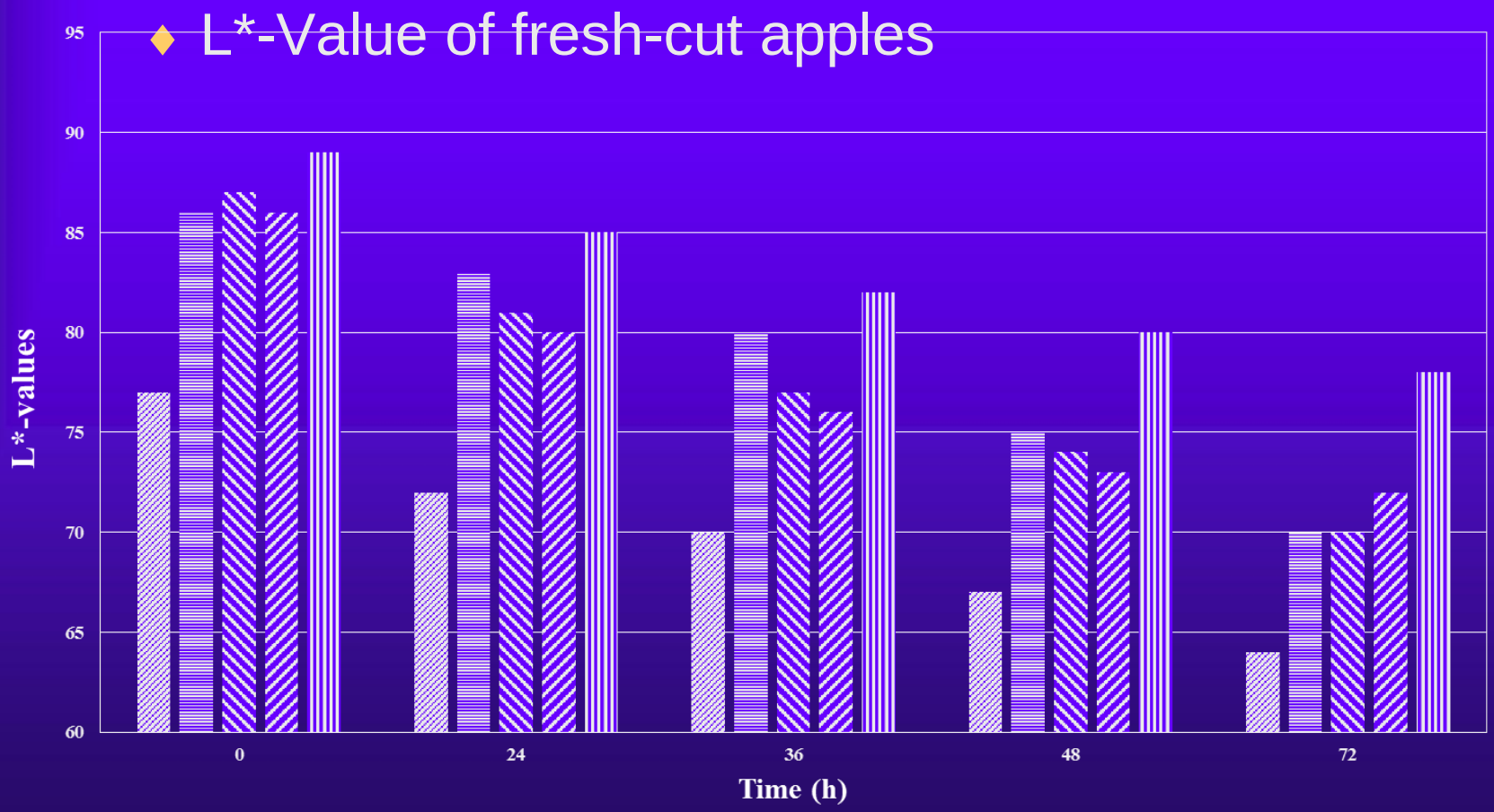
Inhibition of oxidation Rxn

Inhibition of browning Rxn

Supplemental Results; Inactivation of bacteria on fresh-cut pieces



Changes in color of treated & untreated fresh-cut pieces



For more information

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Biocontrol interventions for inactivation of foodborne pathogens on produce at post-harvest

Modesto Olanya

USDA-Agricultural Research Service
Food Safety & Intervention Technology Research Unit, Eastern Regional Research Center, Wyndmoor, PA

Presentation Outline

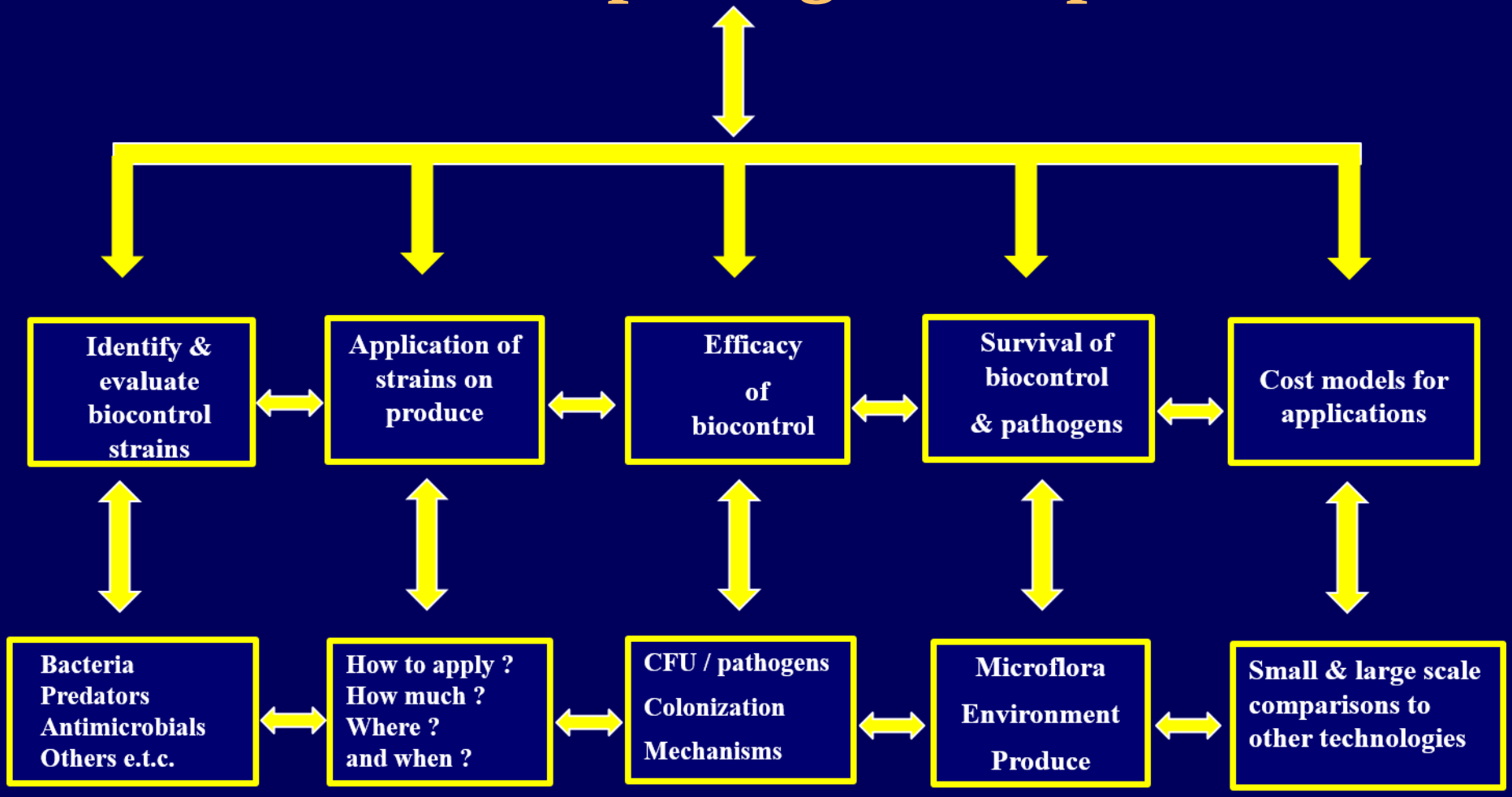
- Broad objectives of biocontrol research for produce safety.
- An overview of factors for development of biocontrol interventions.
- Biocontrol efficacy of *E. coli* O157:H7 with microbial antagonist (*P. fluorescens*) based on competitive exclusions.
- Biocontrol potential of *S. enterica* with predatory bacteria on produce.
- Summary remarks on biocontrol potential combined with gas-phase antimicrobials, physical measures & future research.

Research Objectives

- Develop and evaluate novel antagonists for biocontrol-based interventions of foodborne pathogens on produce.

- Identify combinations of biocontrol measures with physical and chemical interventions for effective inactivations of food-borne pathogens.

Biocontrol interventions for inactivation of foodborne pathogens on produce



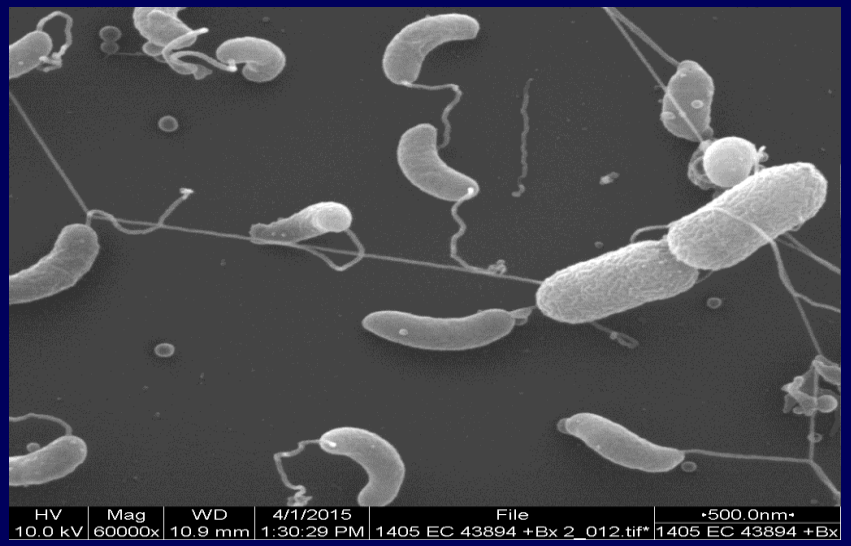
Efficacy of *P. fluorescens* on *E. coli* O157:H7 (*Ec*) inoculated on spinach (20 ºC)

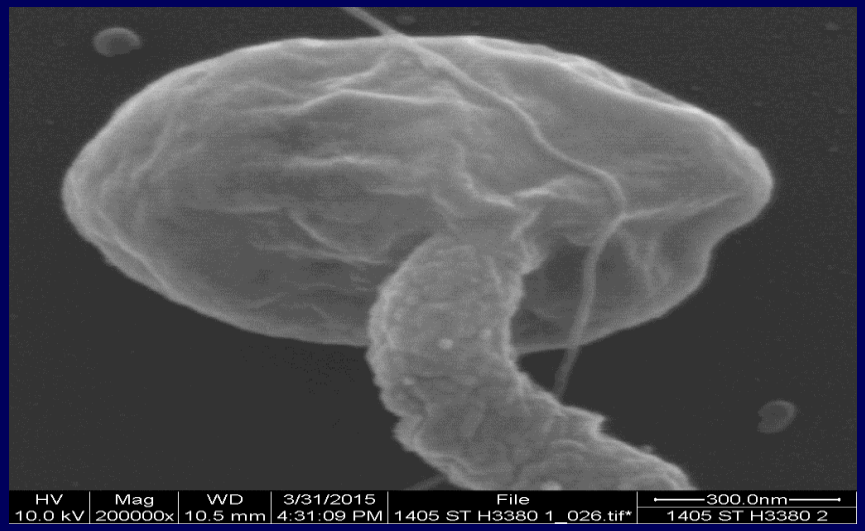
Treatment	24 hrs
P. fluorescens (*Pf*) and *Ec*	Reduction of *Ec* (Log CFU/g)
***Ec* 43894 + *Pf* 2-79**	0.95±0.45b
***Ec* 43894 + *Pf* Q287**	2.10±0.04a
***Ec* 43894 + *Pf* Q8R-1**	1.60±0.00a
***Ec* 43895 + *Pf* 2-79**	1.05±0.65b
***Ec* 43895 + *Pf* Q287**	1.50±0.20ab
***Ec* 43895 + *Pf* Q8R-1**	0.80±0.16b

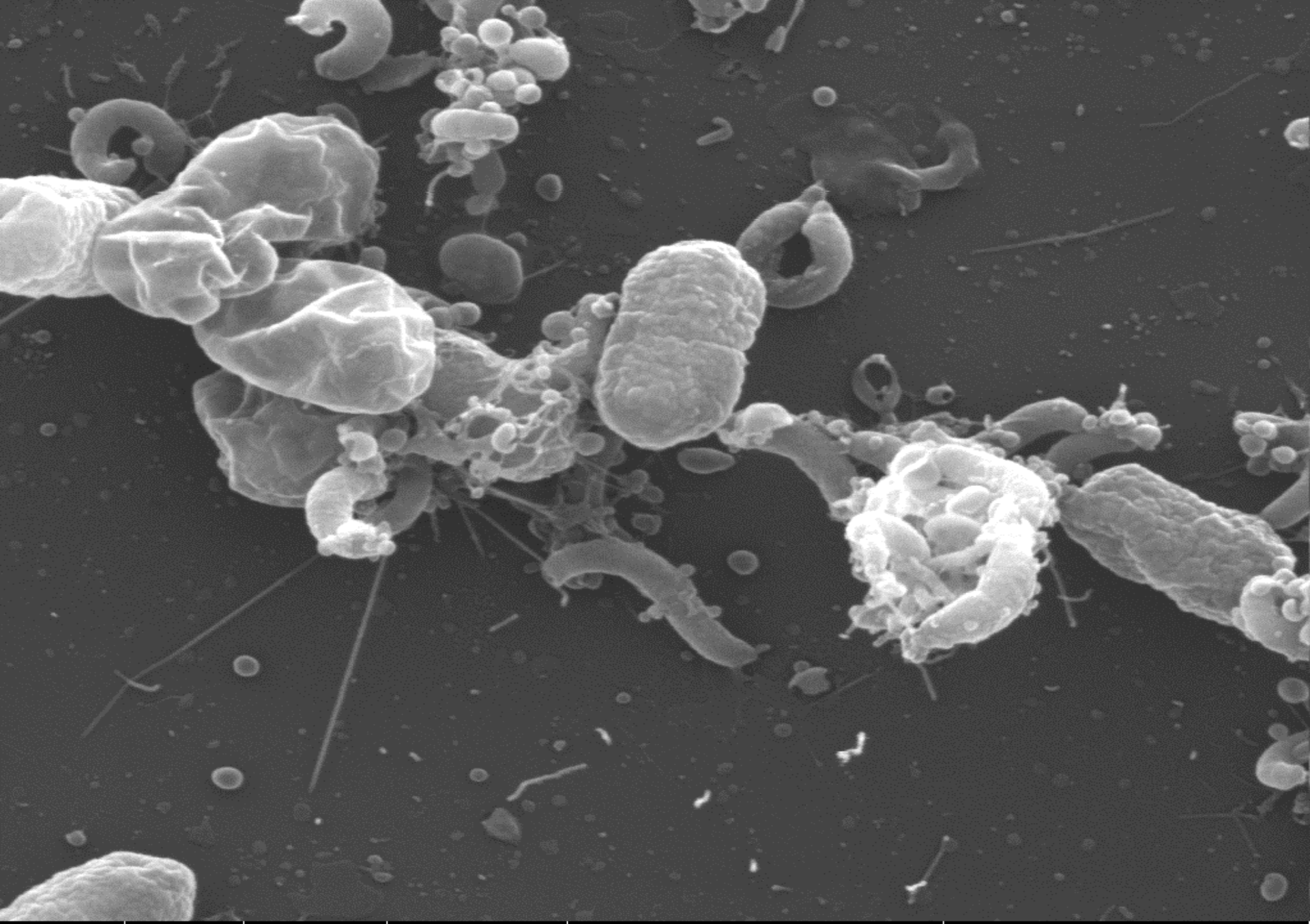
Means with the same letters are not significantly different ($P<0.05$) .

Predatory prokaryotes & bacteria predation

Parameters	*Bdellovibrio / Halobacteriovorax*
Occurrence	Seawater, soil, water
Size & morphology	0.35 x 1.2 µm, curved rods
Motility	Single polar sheath flagellum
Site in prey	Periplasmic space
Reproduction	Bdelloplasts, segmentation
Prey hosts	Gram negative bacteria
Host specificity	Obligate / host independent forms



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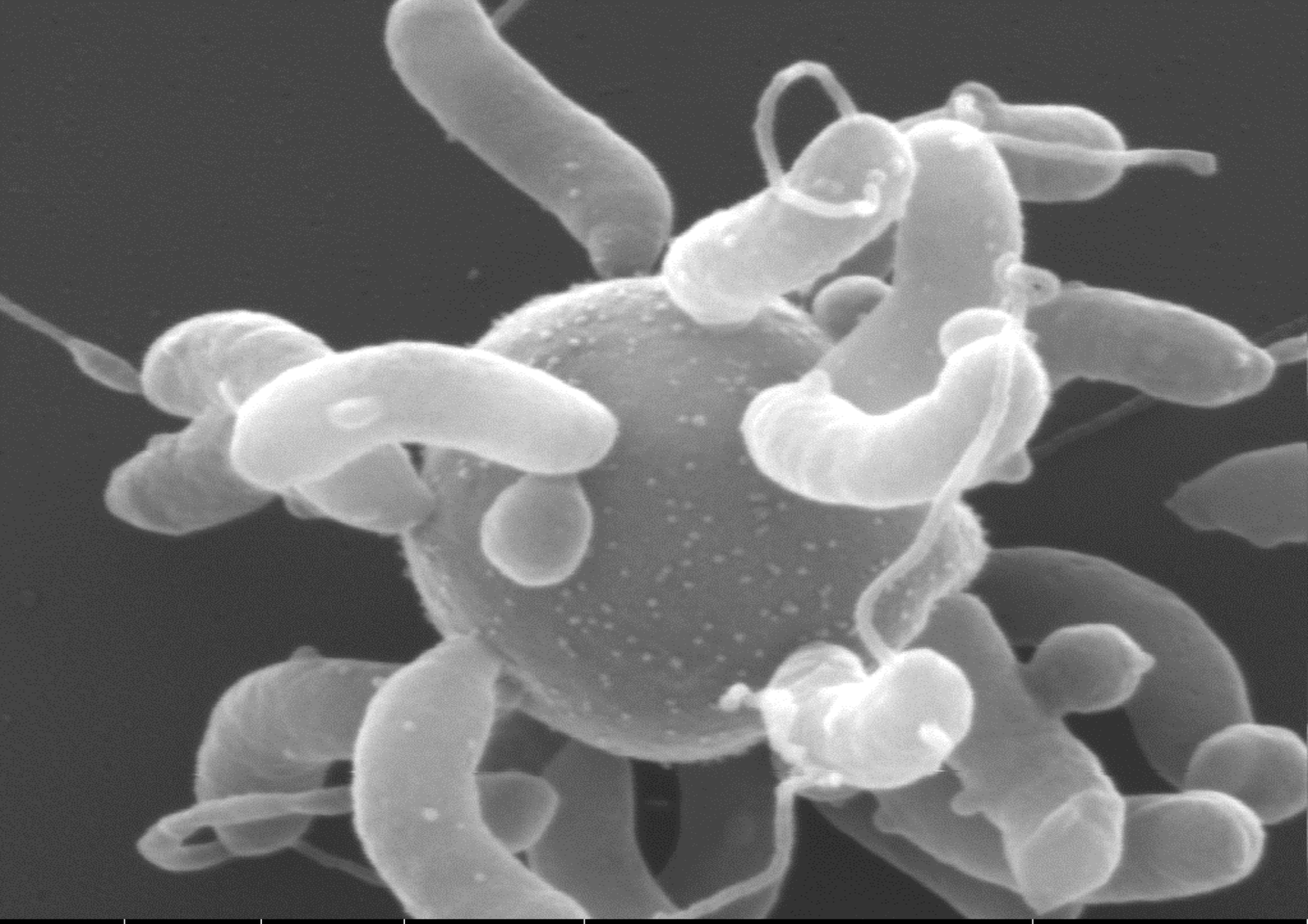
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2380

Predatory *Halobacteriovorax* (*Hbvx*) effects on the survival of *S. enterica* Typhimurium (2380) in seawater and produce

Substrates / Produce	*S. enterica* (control) (Log CFU/g)	*S. enterica* + *Hbvx* (Log CFU/g)	*Hbvx* Plaques (PFU/ml)
Seawater	7.83±0.17a	5.51±0.07b (2.32)	$55x10^3$ PFU/ml
Grape tomatoes	6.19±0.21a	5.18±0.27b (1.01)	$3x10^3$ PFU/ml
Clover sprouts	6.15±0.11a	4.98±0.13b (1.17)	$20.3x10^3$ PFU/ml
Lettuce	6.68±0.12a	6.47±0.15a (0.21)*ns*	$12.6x10^3$ PFU/ml

Assayed after 24 hrs. Means across rows with the same letters are not significantly different (*P<0.05*).

***Chlorine dioxide was applied at 0.4 mg/L, 90% R.H., and 13 °C.**


QUESTIONS?
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Development of decontamination technologies for fresh and minimally processed produce

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Research Microbiologist

Food Safety and Intervention Technologies Research Unit

Eastern Regional Research Center, USDA-ARS

Wyndmoor, PA

February 23, 2017

Why

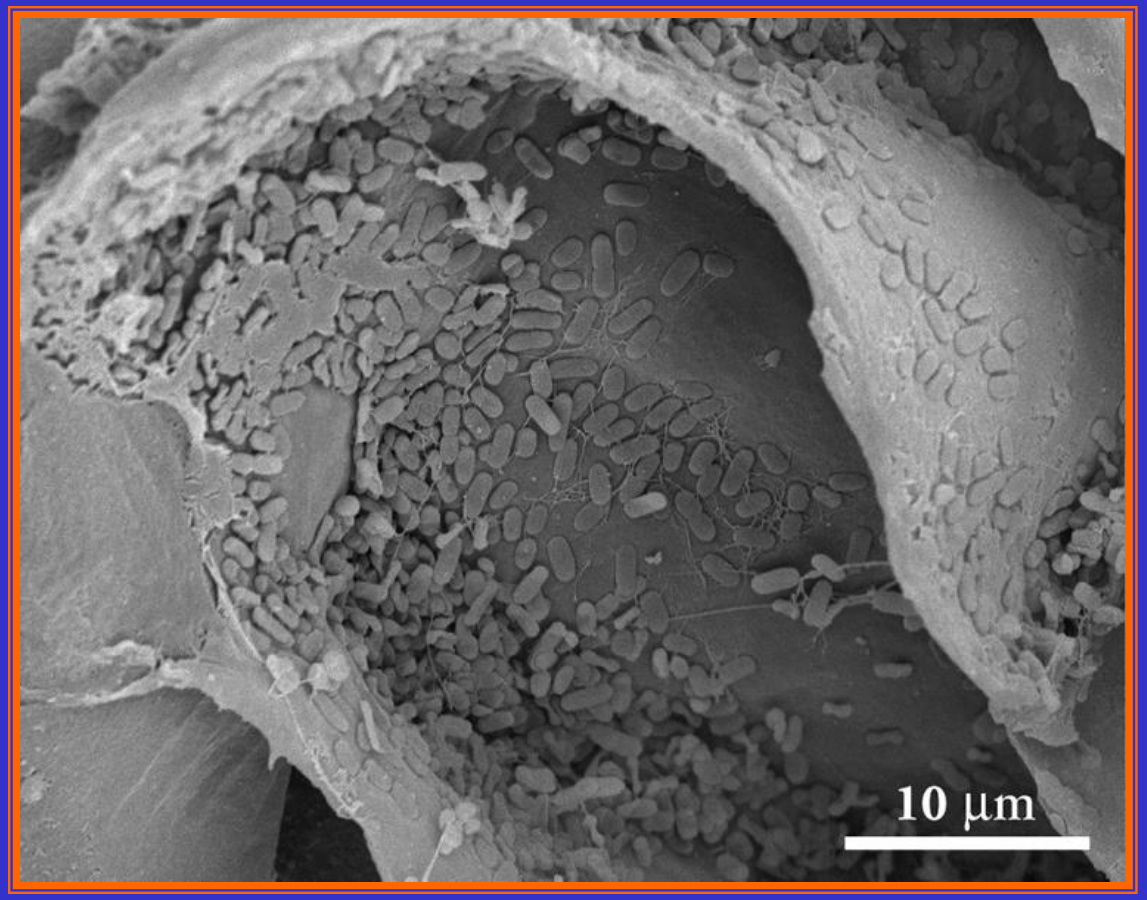
The lack of efficacy using aqueous sanitizers was attributed to bacterial attachment to inaccessible sites on the cantaloupe rind and/or biofilm formation

(Annous et al., 2004. *J. Food Prot,* 67: 1876-1885)

Cantaloupe surfaces: a tangled net

- Epidermal cell surface ruptured by meshwork of raised tissue (netting)
- Netting consists of lenticels and phellum
- Guard cells non-functional
- Cuticular cracks-points of ingress for microorganisms

SEM (2500X) of *S.* Poona Cells Inside the Netting



2 h drying at RT

Personal Research Projects

- Thermal Treatment of cantaloupe, tomatoes, and sprouting seeds
- Chlorine dioxide gas treatments
- Anti-microbial Packaging systems
- Rapid detection of produce contamination
- BSL-2 Pilot Plant Processing facility

Residual populations of **Salmonella** *Poona on artificially inoculated cantaloupes stored for 24 h at 4ºC or room temperature*

Treatment[c]	4ºC (log CFU/cm^2)		Room temperature (log CFU/cm^2)	
	XLT-4	TSA with XLT-4 overlay	XLT-4	TSA with XLT-4 overlay
2 h Control	4.81 ± 0.43 A	5.40 ± 0.42 A	5.11 ± 0.44 AB	5.76 ± 0.50 AB
24 h Control	4.18 ± 0.56 AC	4.95 ± 0.33 AC	6.19 ± 0.31 D	6.59 ± 0.50 AD
24 h 200 ppm chlorine (RT for 20 min)	3.36 ± 0.15 C	3.79 ± 0.03 C	5.30 ± 0.39 BD	5.74 ± 0.42 BD
24 h Acidic electrolyzed water (RT for 20 min)	3.20 ± 0.22 C	3.85 ± 0.10 C	5.70 ± 0.04 BD	5.88 ± 0.08 BD
24 h Basic electrolyzed water (RT for 20 min)	3.57 ± 0.60 C	4.25 ± 0.50 AC	5.83 ± 0.58 BD	6.01 ± 0.63 BD
24 h tap water (76ºC for 3 min)	0.04 ± 0.02[d] E	0.04 ± 0.02[d] E	1.15 ± 0.63[d] E	1.48 ± 0.53[d] E
24 h tap water (RT for 20 min)	3.22 ± 0.37 C	4.59 ± 0.38 AC	5.87 ± 0.51 BD	6.15 ± 0.56 BD

SEM

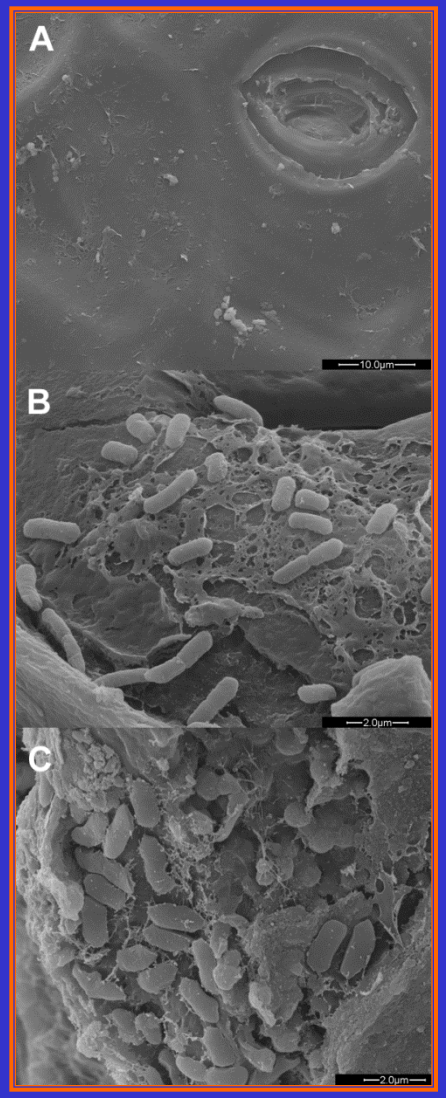
A) non-inoculated sample

B) inoculated sample

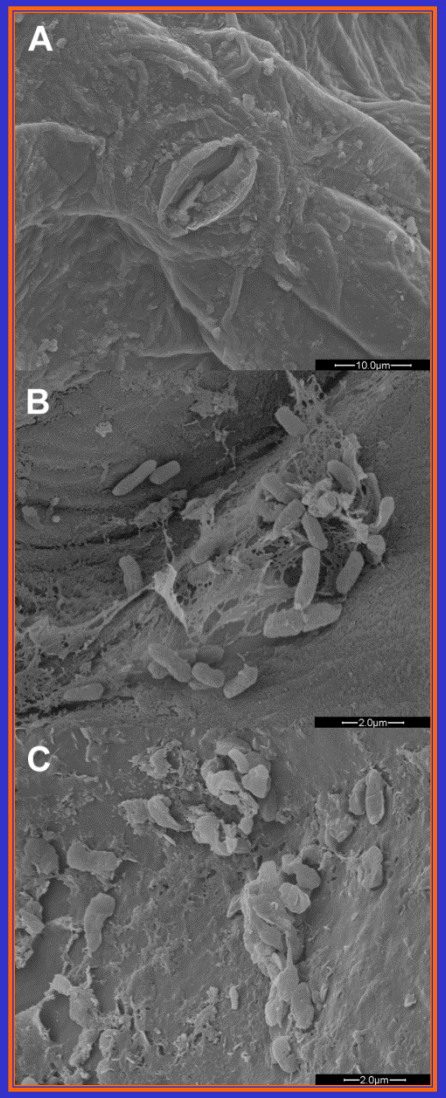
C) inoculated and treated

(Technology transferred to industry)

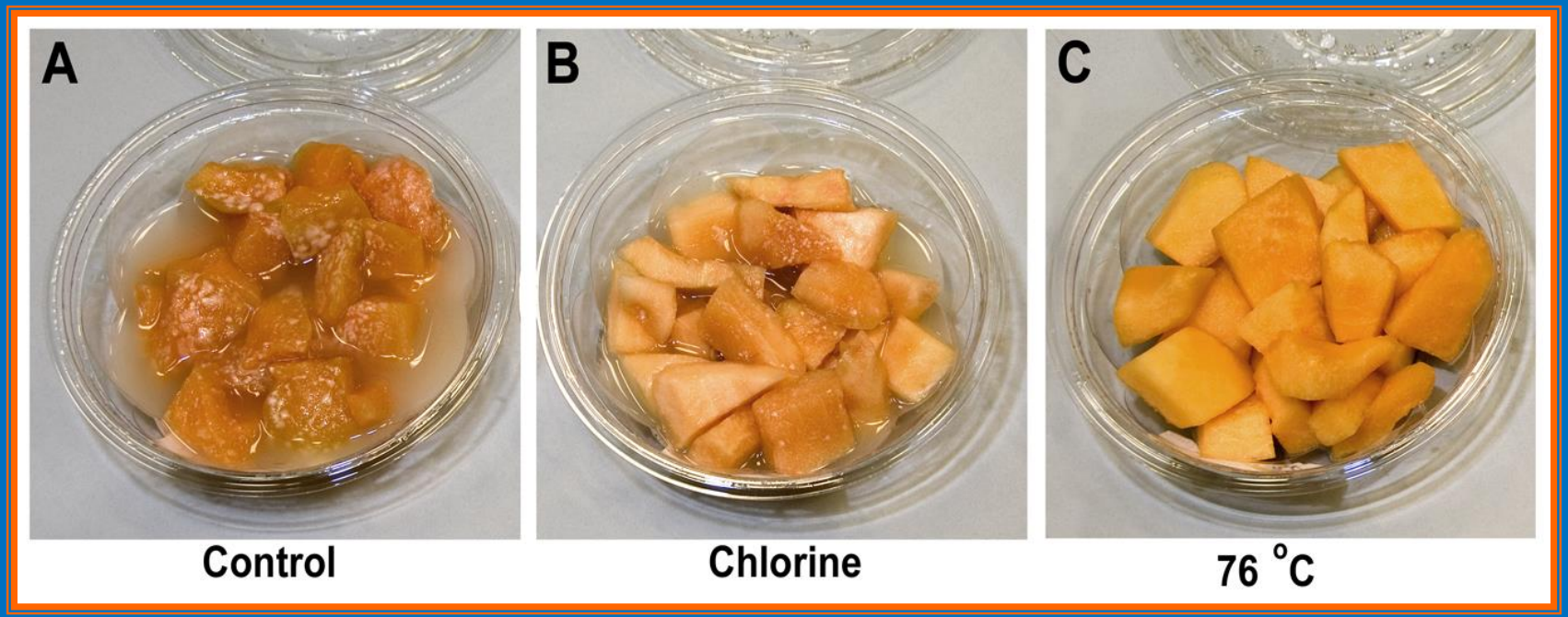
Stem Scar



Rind



Fresh-cut prepared from treated cantaloupes and stored at 4°C for Two Months



Efficacy of hot water vs. chlorine wash on inactivating *Salmonella* Poona on green tomatoes

Treatment	4ºC (log CFU/gm)		Room Temperature (log CFU/gm)	
	XLT4	TSA-XLT4	XLT4	TSA-XLT4
2 h Control	3.47± 0.20	4.31 ± 0.25	3.69 ± 0.17	4.60 ± 0.07
24 h Control	2.84 ± 0.73	3.85 ± 0.83	5.76 ± 0.60	5.93 ± 0.55
20 ppm Cl_2	2.20 ± 0.48	3.18 ± 0.68	4.65 ± 0.31	5.10 ± 0.45
Tap water at 70ºC 3.5 min	0.97 ± 1.19 (8/12)	1.07 ± 1.17 (7/12)	0.70± 0.66 (8/12)	0.79 ± 0.79 (8/12)

Efficacy of hot water treatments (90 s) in reducing *Salmonella* on mung bean seeds

Treatment Temperature (°C)	Log CFU/gm seed	Log CFU/ gm sprout	% Germination
No Treatment	5.44 ± 0.12	8.24 ± 0.10	97
70	0.30 ± 0.00	7.32 ± 0.14	98
75	No Growth	7.11 ± 0.55	96
80	No Growth	No Growth	95
90	No Growth	No Growth	25

Control

Treated

- Shredded lettuce + *E coli* O157:H7 + ClO_2 (Patented)

- Stored at 4^{o}C

Biobased Insert (Patent pending)

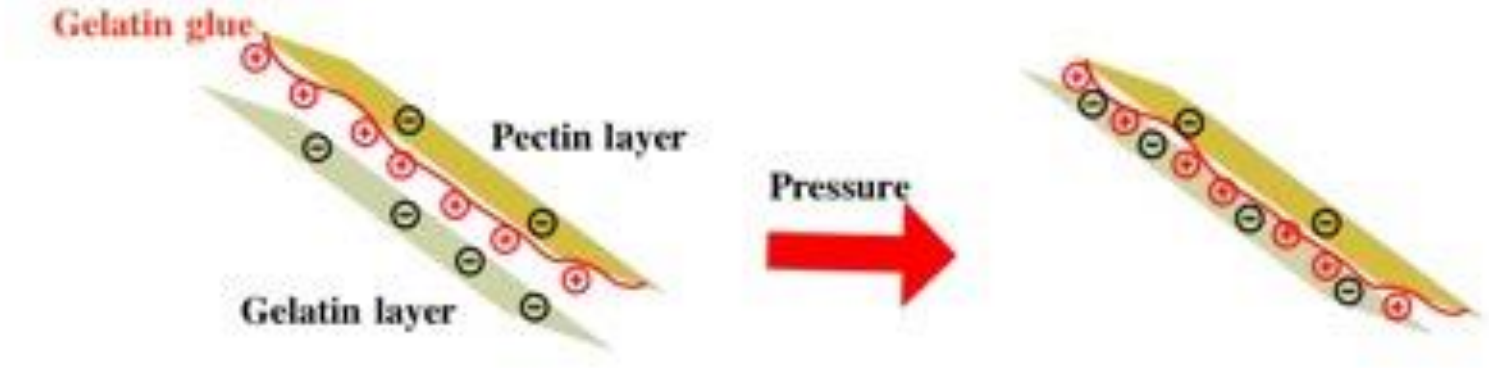


Figure 2. Inter-layer binding of pectin and gelatin layers.
Application of the positively charged gelatin glue solution on the surface of slightly negatively charged pectin followed by pressing onto the negatively charged gelatin layer to promote binding and achieve contact between the layers.

Multi-Layer Insert

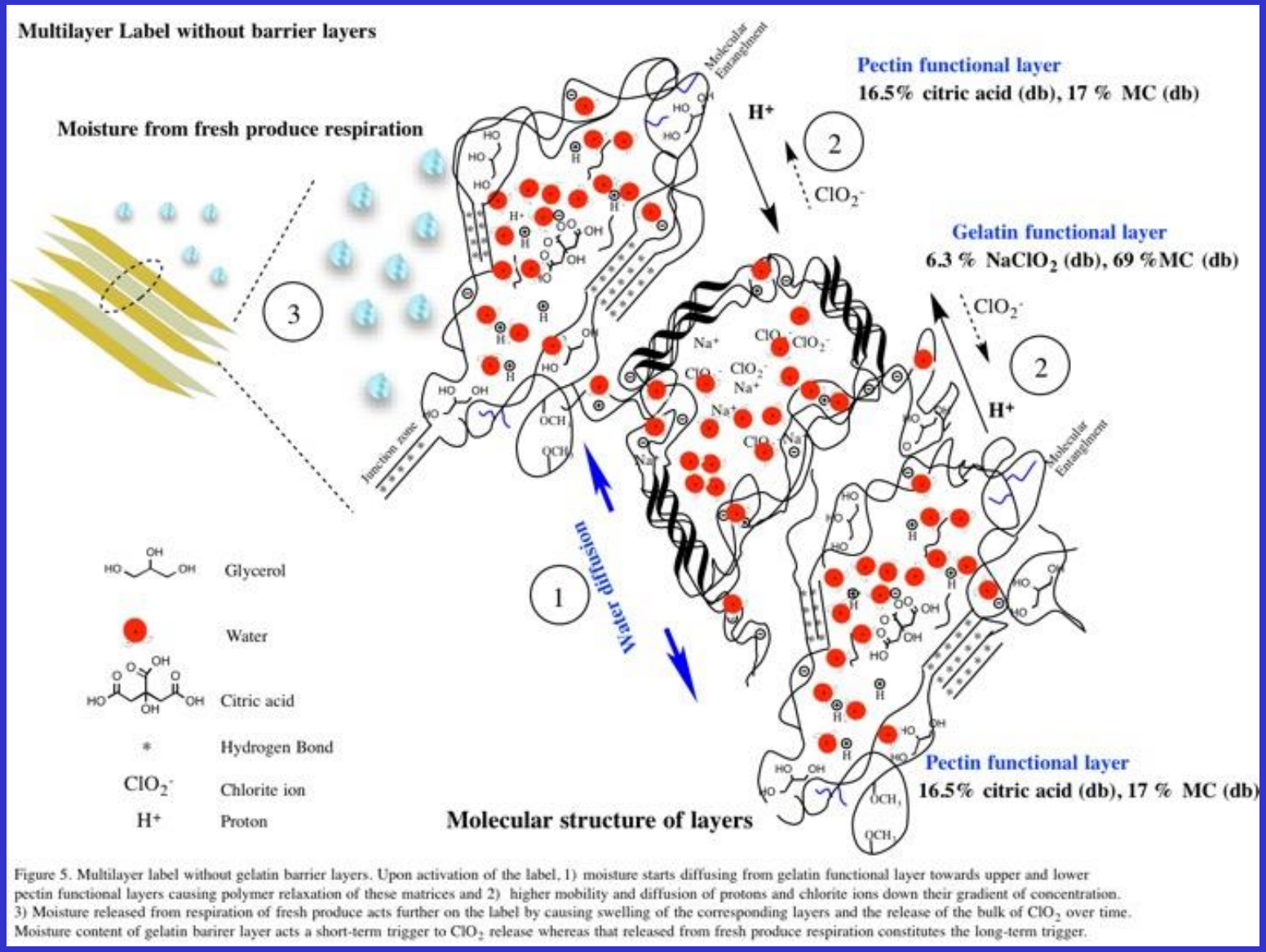


Figure 5. Multilayer label without gelatin barrier layers. Upon activation of the label, 1) moisture starts diffusing from gelatin functional layer towards upper and lower pectin functional layers causing polymer relaxation of these matrices and 2) higher mobility and diffusion of protons and chlorite ions down their gradient of concentration. 3) Moisture released from respiration of fresh produce acts further on the label by causing swelling of the corresponding layers and the release of the bulk of ClO_2 over time. Moisture content of gelatin barirer layer acts a short-term trigger to ClO_2 release whereas that released from fresh produce respiration constitutes the long-term trigger.

Thank You

ERRC

EASTERN REGIONAL RESEARCH CENTER

Cold plasma as a novel inter vention against food-borne pathogens

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Cold Plasma

- **Cold plasma (CP)** – ionized gas containing reactive species, including ozone, UV photons, free radicals, positive/negative ions, electrons, atoms
- **Non-thermal preservation method**
- **Inputs** –

  Energy (electricity, microwaves, etc.)

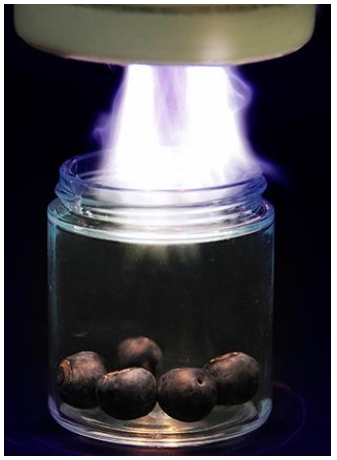
  Plasma-forming gas (He, O_2, N_2, air etc.)
- **Variables** –

  Processing: electrical variables, energy level, treatment time, gas variables, etc.

  Food: composition, size (volume, height), surface characteristics

  Microorganisms: Species/isolate, growth phase, vegetative cells/spores, etc.

  Packaging: packaging materials, size, etc.



Ongoing ERRC cold plasma research

- Gliding arc plasma, DBD – surface treatments of bulk foods
  - →Norovirus, norovirus surrogates
  - →*E. coli* O157:H7, *Salmonella, L. monocytogenes*
- Vacuum process cold plasma system
- In-package treatment of salad vegetables
- Flow-through treatment of dry flowable foods
- Combinations with other antimicrobial interventions
  - → High-intensity light
  - → Biological controls
  - → Precision thermal, irradiation
- R&D partnerships with industry
- NIFA research grants with cold plasma
  - → University of Maine ($5M, 5-year), through 2019

Cold plasma

In-package generation of cold plasma as an antimicrobial process

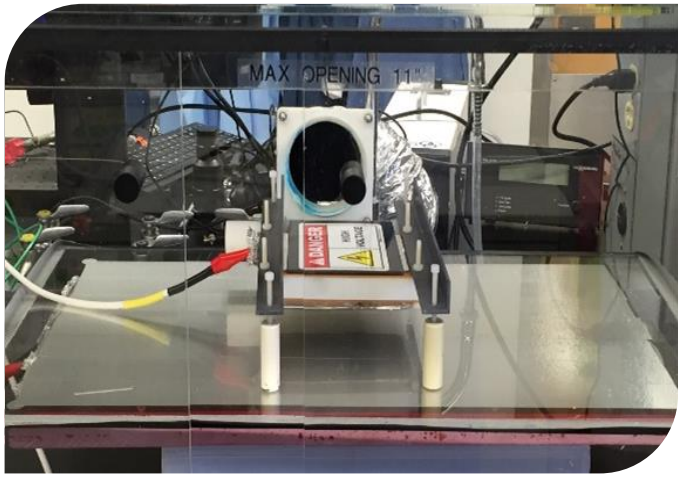
Packaging preparation and treatment

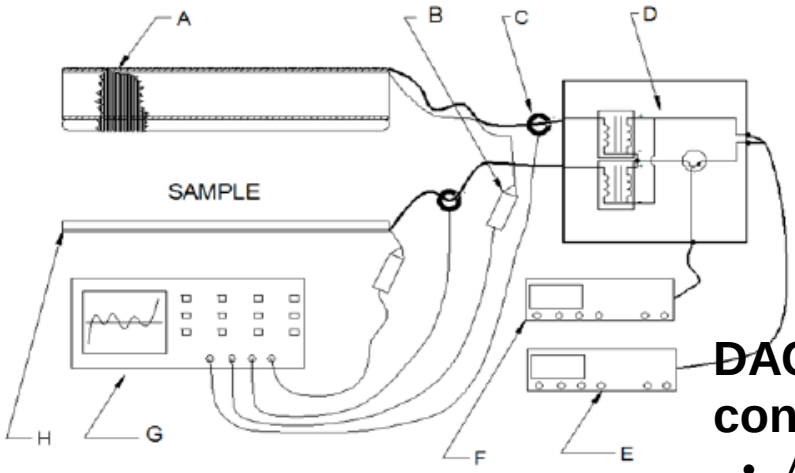
Rigid test container

Plastic bag

Plastic bag with water vaporization

Commercial clamshell container





DACP treatment conditions -

- Applied voltage: 38–70 kV
- Current: 1–3 A

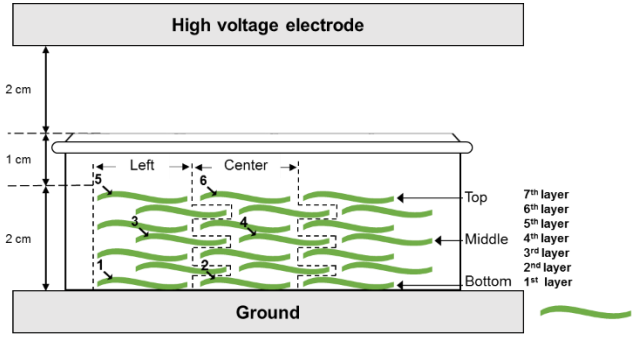
Results – Viral reduction

Effects of in-package DACP treatment with MAP, and post-treatment cold storage on the microbial inactivation of Tulane virus

Treatment	Package	Viral reduction (log PFU/g lettuce)
		Tulane virus
DACP treatment	Rigid	1.1 ± 0.3
	Flexible	1.3 ± 0.1
DACP + Post-treatment storage, 24h at 4°C	Rigid	1.3 ± 0.2
	Flexible	0.5 ± 0.2
5% O2 (balance N2) MAP2 + DACP	Flexible	0.7 ± 0.3
10% O2 (balance N2) MAP + DACP	Flexible	0.1 ± 0.2

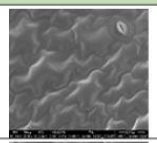
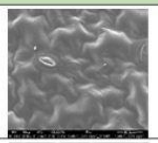
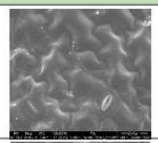
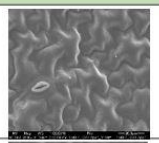
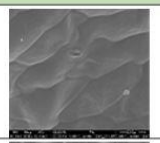
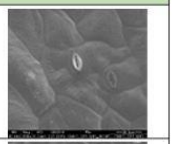
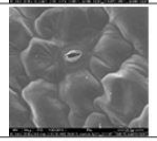
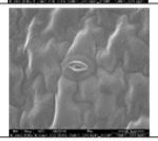
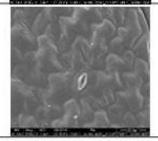
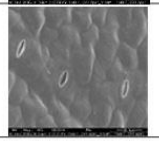
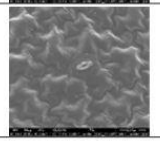
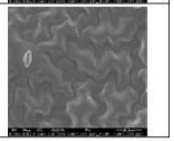
* Treatment voltage, current, and treatment time were 47.6 kV, 1 A, and 5 min, respectively.

Cold plasma - bulk lettuce preparation



Schematic diagram of the cold plasma reactor holding a clamshell package treatment sample containing seven layers of lettuce leaves in a three-column configuration.

Position		Microbial reduction (log CFU/g lettuce)				
		1-layer configuration	3-layer configuration	5-layer configuration	7-layer configuration	
					No shaking	Shaking
Top	Left		0.6 ± 0.4 ab	0.6 ± 0.4 ab	0.9 ± 0.2 a	0.7 ± 0.2 ab
	Center		0.6 ± 0.1 ab	0.6 ± 0.3 ab	1.1 ± 0.1 a	0.7 ± 0.2 ab
Middle	Left		0.6 ± 0.3 ab	0.5 ± 0.2 ab	0.6 ± 0.1 ab	0.8 ± 0.2 ab
	Center		0.7 ± 0.4 ab	0.8 ± 0.3 ab	0.6 ± 0.2 ab	0.6 ± 0.1 ab
Bottom	Left	0.6 ± 0.2 ab[b]	0.5 ± 0.3 ab	0.5 ± 0.3 ab	0.4 ± 0.1 b	0.7 ± 0.1 ab
	Center	0.7 ± 0.2 ab	0.6 ± 0.1 ab	0.5 ± 0.3 ab	0.4 ± 0.1 b	0.7 ± 0.1 ab

Sample location		Top		Middle		Bottom	
		Untreated	DACP-treated	Untreated	DACP-treated	Untreated	DACP-treated
Top	Left						
	Center						

Conclusions

- *E. coli O157:H7* and *L. monocytogenes* had comparable responses to DACP, *Salmonella* was less sensitive.
- DACP reduced the initial counts of *E. coli* O157:H7, *L. monocytogenes*, and Tulane virus by 1.1 log CFU/g, 1.0 log CFU/g, and 1.3 log PFU/g, respectively, in a non-thermal treatment manner.

  → DACP has potential for use as a post-packaging microbial decontaminating process for lettuce products
- The reduced-oxygen MAP used in the present study decreased the inactivation rates of *E. coli* O157:H7 and Tulane virus.

Conclusions

- The *L. monocytogenes* population declined by an additional 0.6 log CFU/g after being stored at 4 °C for 24 h

  → Cold storage can enhance the treatment effect against *L. monocytogenes*

- For bulk packaged lettuce, inhibition rates of *E. coli* O157:H7 were consistent in all locations for 1-, 3-, 5-, and shaken 7-leaf stacks.
- Both the rigid and flexible types of conventional and commercial plastic packages can be directly used for packaging lettuce that will be decontaminated by DACP

  → Current produce packaging lines can be utilized with DACP processing, with further optimization

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In-package inhibition of *E. coli* O157:H7 on bulk Romaine lettuce using cold plasma

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Atmospheric cold plasma inactivation of aerobic microorganisms on blueberries and effects on quality attributes

Alison Lacombe [a], Brendan A. Niemira [a, *], Joshua B. Gurtler [a], Xuetong Fan [b], Joseph Sites [a], Glenn Boyd [a], Haiqiang Chen [c]

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Research Paper

Inactivation of *Escherichia coli* O157:H7 and Aerobic Microorganisms in Romaine Lettuce Packaged in a Commercial Polyethylene Terephthalate Container Using Atmospheric Cold Plasma

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Nonthermal inactivation of norovirus surrogates on blueberries using atmospheric cold plasma☆

Alison Lacombe [a], Brendan A. Niemira [b, *], Joshua B. Gurtler [b], Joseph Sites [b], Glenn Boyd [b], David H. Kingsley [c], Xinhui Li [d], Haiqiang Chen [e]

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Dielectric barrier discharge atmospheric cold plasma inhibits *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, and Tulane virus in Romaine lettuce☆

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