Non-Thermal Processing Technologies Research at the USDA's Eastern Regional Research Center

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Research Leader

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http://www.tinyurl.com/FSIT-RU

Food Safety and Intervention Technologies Research Unit

- Microwave, infra-red, radio frequency
- Light: UV, pulsed, high-intensity monochromatic
- High pressure processing
- Flash steam treatment
- Ozonation, ClO_2, MAP
- GRAS and novel antimicrobials
- Irradiation
- Cold plasma
- Biocontrols: predators, competitors, antagonists, bacteriophage

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Irradiation

Why irradiation?

- Investigated for 100+ years. Developed into a safe, effective food processing technology
  - Shelf life extension
  - Sprout suppression
  - Antimicrobial efficacy – spoilage and safety
- GAP, GMP, preventative controls are effective, but are only part of the answer
- Chemical sanitizers and physical controls
  - Limitations of use, efficacy
- Alternative and supplemental interventions

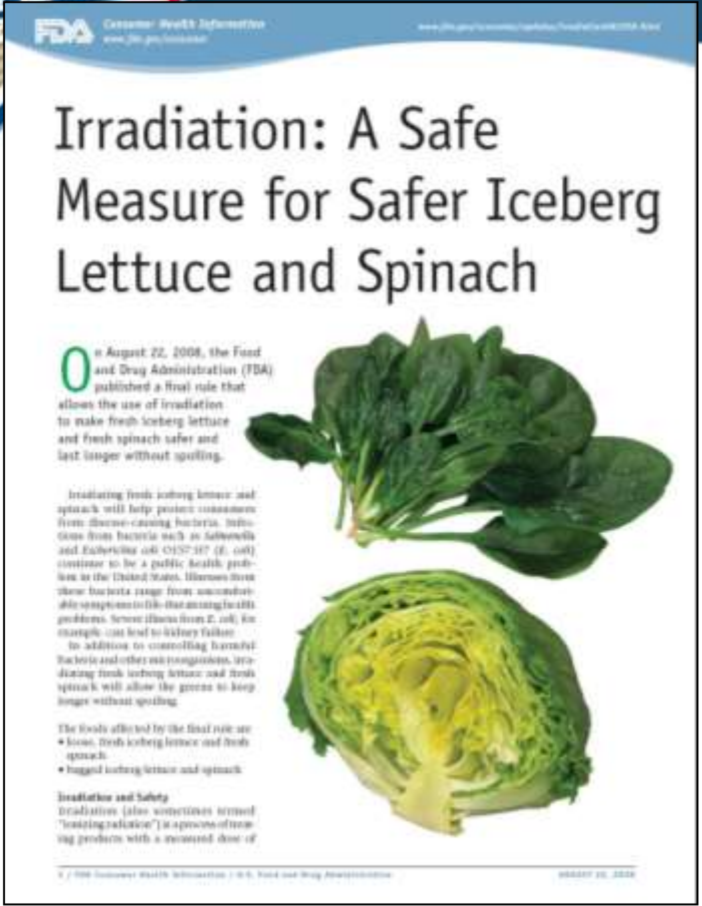
Mode of action

- Largest target in organisms is water
- High energy electrons break water molecules into OH• and O• radicals, which disrupt membranes, proteins and nucleic acids
- DNA is also broken directly
- High energy photons interact with atoms to eject high energy electrons
- Penetration of photons is much greater than for electrons - implications for how material is processed

Irradiation applications

- Prevention of foodborne illness – e.g. *Salmonella* and *Escherichia coli*
- Preservation – inactivate spoilage organisms, extend shelf life
- Phytosanitation – destroy insects, larvae, eggs in or on tropical imports, decreasing need for more harmful pest-control practices
- Delay of sprouting and ripening – e.g. potatoes, onions, bulbs
- Sterilization – shelf-stable, sterile packaged foods. Used for hospitals, military, astronauts
- Irradiation equally effective against **antibiotic resistant** P. aeruginosa, E. coli O157:H7 (Dharmarha et al., IAFP 2018)

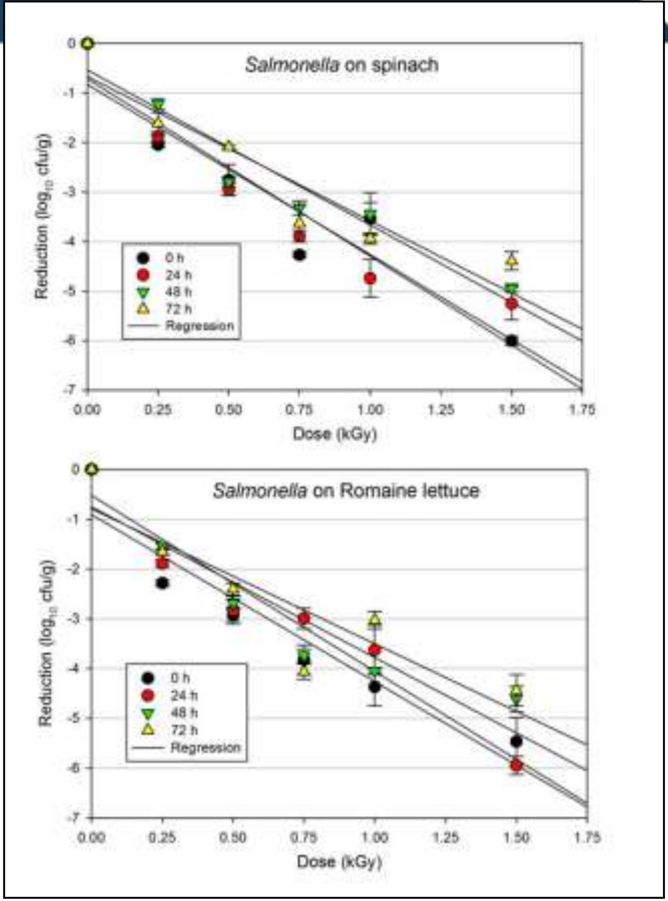
Irradiation – leafy greens



Consumer Health Information

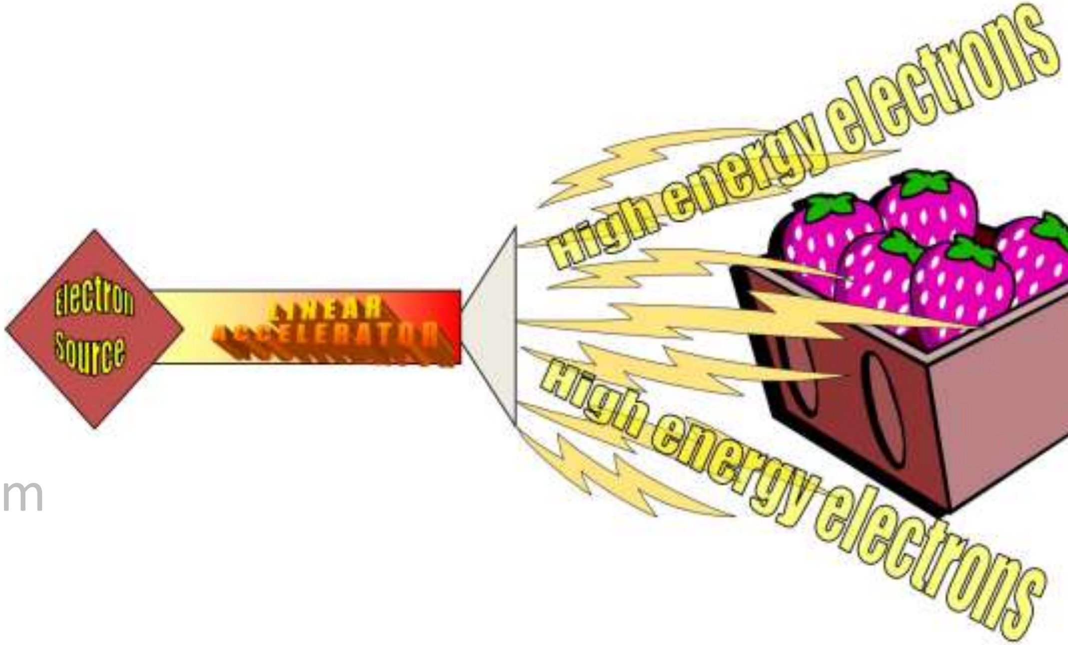
Irradiation: A Safe Measure for Safer Iceberg Lettuce and Spinach

On August 22, 2008, the Food and Drug Administration (FDA) published a final rule that allows the use of irradiation to make fresh iceberg lettuce and fresh spinach safer and last longer without spoiling.



Technologies: E-beam

- Electron beam
  - Varying designs
  - Linear, cyclotron, rhodotron
  - "On demand"
- X-ray
  - Tungsten, tantalum
  - "On demand"
- Gamma ray
  - "Always on"



Technologies: X-ray

- Electron beam
  - Varying designs
  - Linear, cyclotron, rhodotron
  - "On demand"
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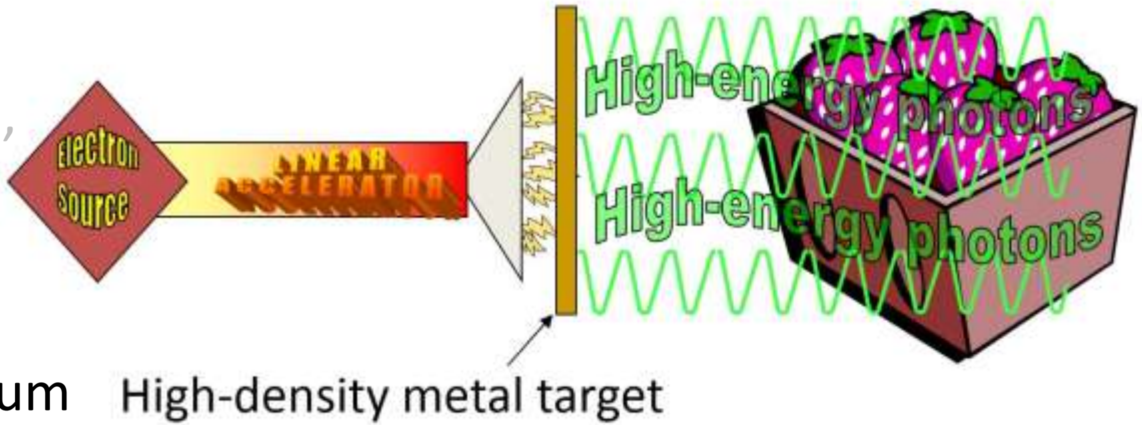
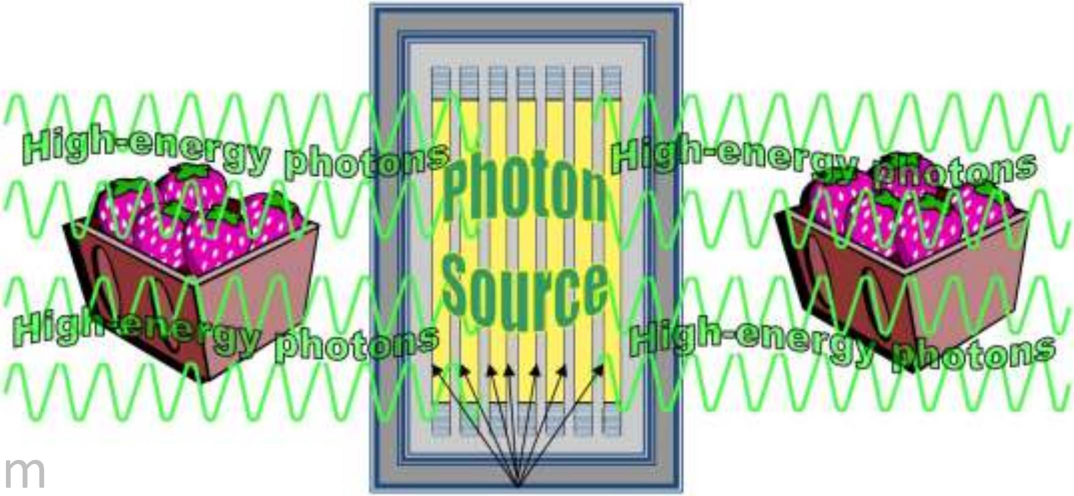


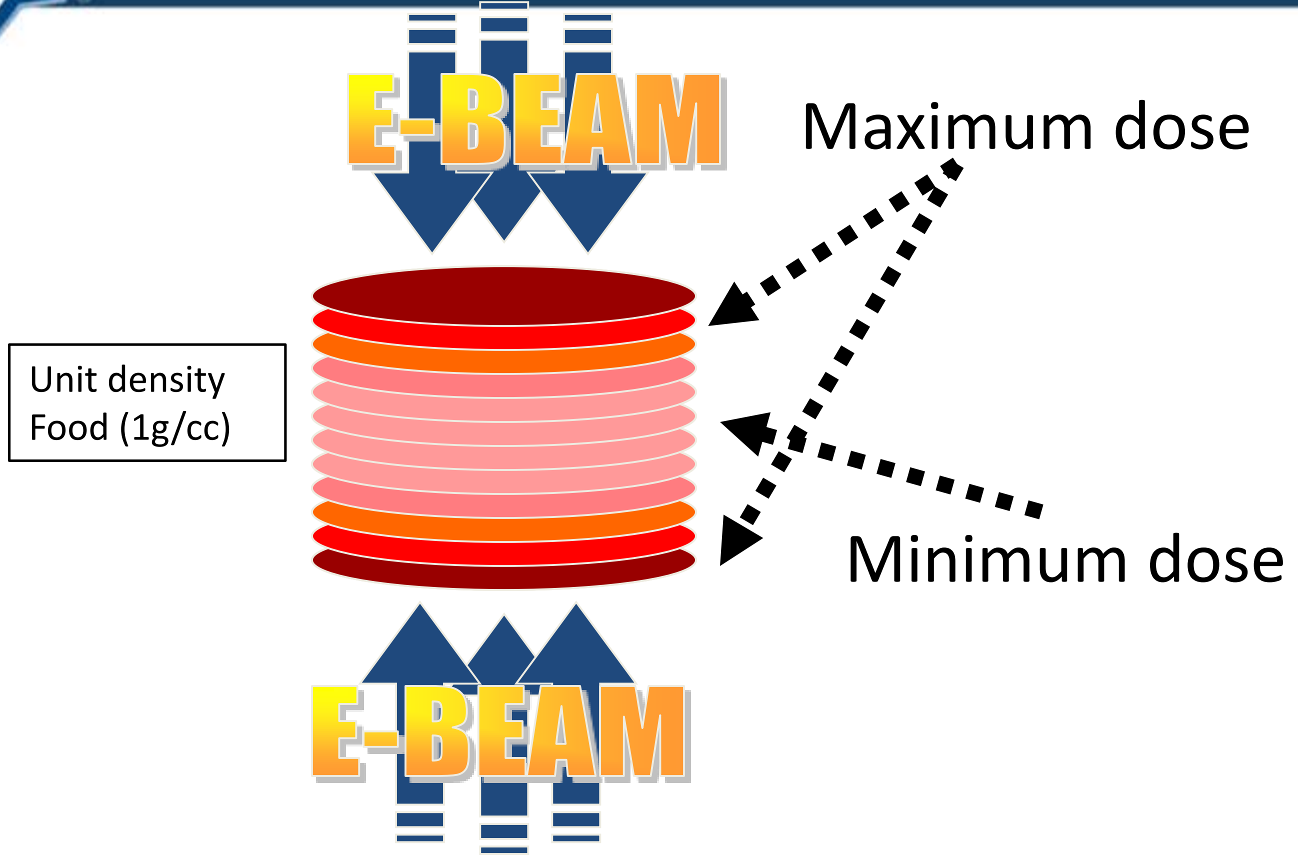
Technologies: gamma

- Electron beam
  - Varying designs
  - Linear, cyclotron, rhodotron
  - "On demand"
- X-ray
  - Tungsten, tantalum
  - "On demand"
- Gamma ray
  - "Always on"

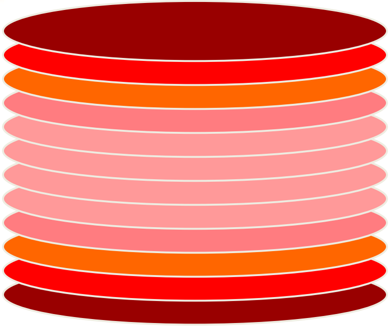


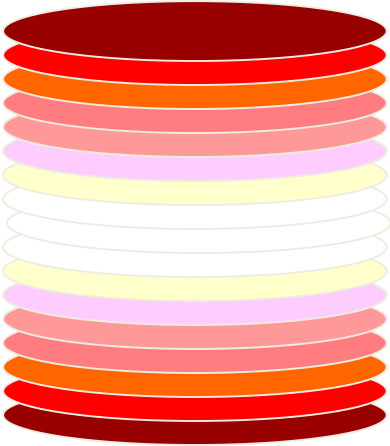
Radioactive material, typically cobalt-60

The Max/Min Ratio



Implications of the Max/Min Ratio





- Good packaging
  - effective penetration of irradiation from above and below
  - relatively even dosage, low Max/Min ratio
- Poor packaging
  - Too bulky, too dense, too thick (or all three)
  - incomplete penetration
  - uneven dosage, high Max/Min ratio

Irradiation – electron beam

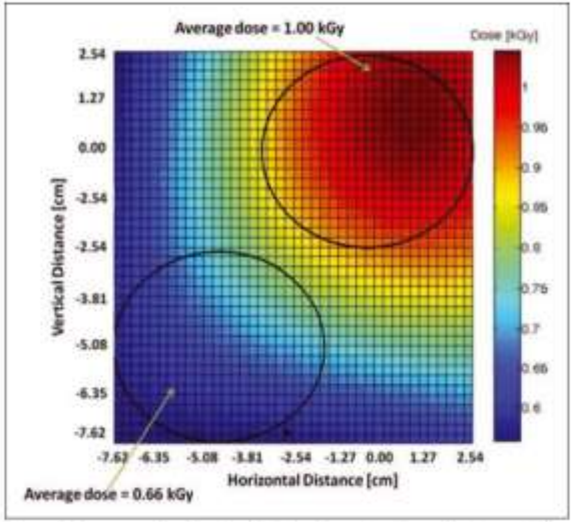


Figure 3–Dose mapping for inoculation of tomato slices with a target dose moved from the calibrated (reference) area by 7.18 cm.

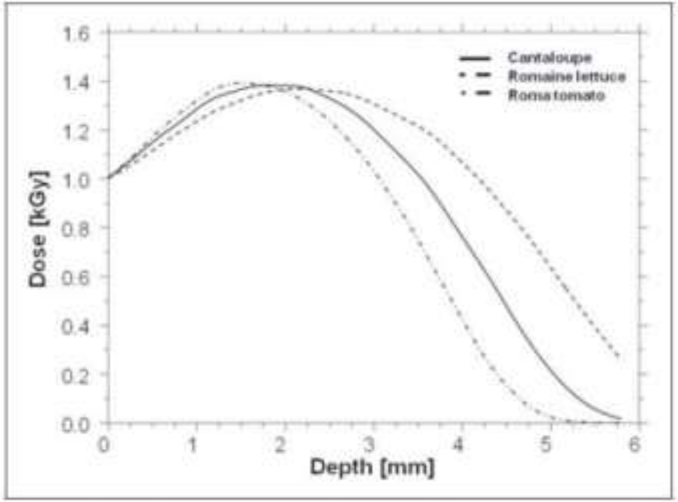


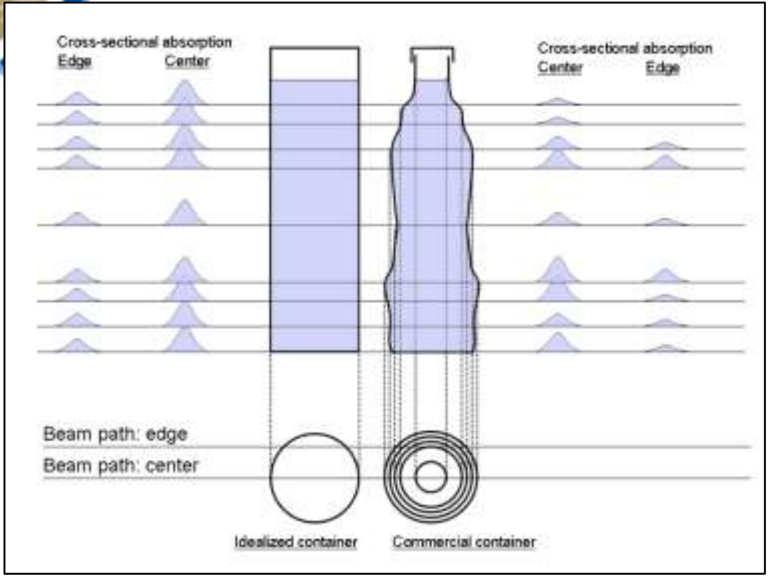
Figure 6–Dose-depth curve for cantaloupe, Romaine lettuce, and Roma tomato irradiated with 1.35 MeV e-beam at 23 °C.

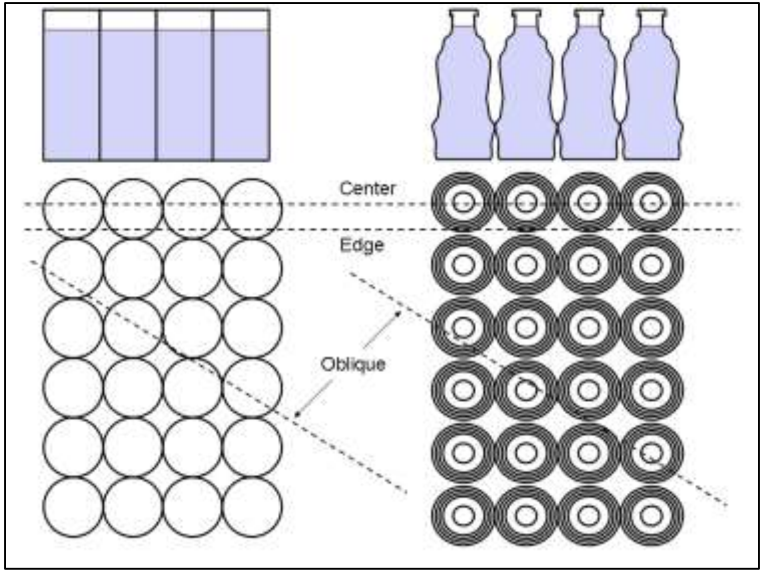
- Dose uniformity is critical. Misalignment of e-beam can reduce absorbed dose from 1.0 kGy to 0.66 kGy
- Product thickness: depth of penetration must be known for each product *(Moriera et al, 2012)*

High Max/Min Ratio: Consequences

- Dose too high = sensory damage
- Dose too low = inadequate kill/control
- Irregularly shaped whole produce can have high Max/Min of ~ 3.0 *(Brescia et al., 2003)*
- Irregular shapes and variations of density common in produce
- Product penetration (one sided)
  - X-ray and gamma, 30-40 cm
  - E-beam, 6-8 cm
- Thick, dense products present a challenge for effective, uniform treatment with e-beam irradiation.
  - Packaging design is critical

Max/Min logistics





- Product orientation with respect to irradiation source
- Individual items vs. commercial bulk packaging (bag, tray, pallet, etc.)

Gamma irradiator

- GrayStar – Co^{60} source
- Below floor level, water shielded
- Process pallet loads in semi-continuous operation

- http://www.graystarinc.com/photos.html

E-beam facility

SPOTLIGHT | April/May 2018

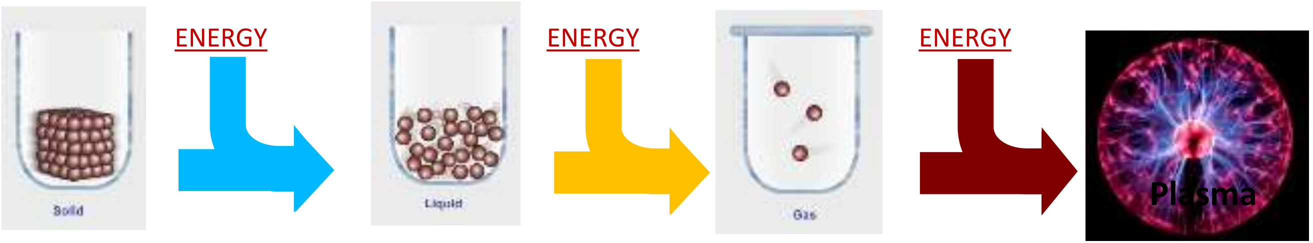
Electron Beam Technology: A Platform for Safe, Fresh, and Chemical-Free Food

By Suresh D. Pillai, Ph.D., and Sohini S. Bhatia

Cold plasma: antimicrobial

Cold plasma

- What is a plasma?
  - "Fourth state of matter"
  - Equivalent to highly energetic ionized gas

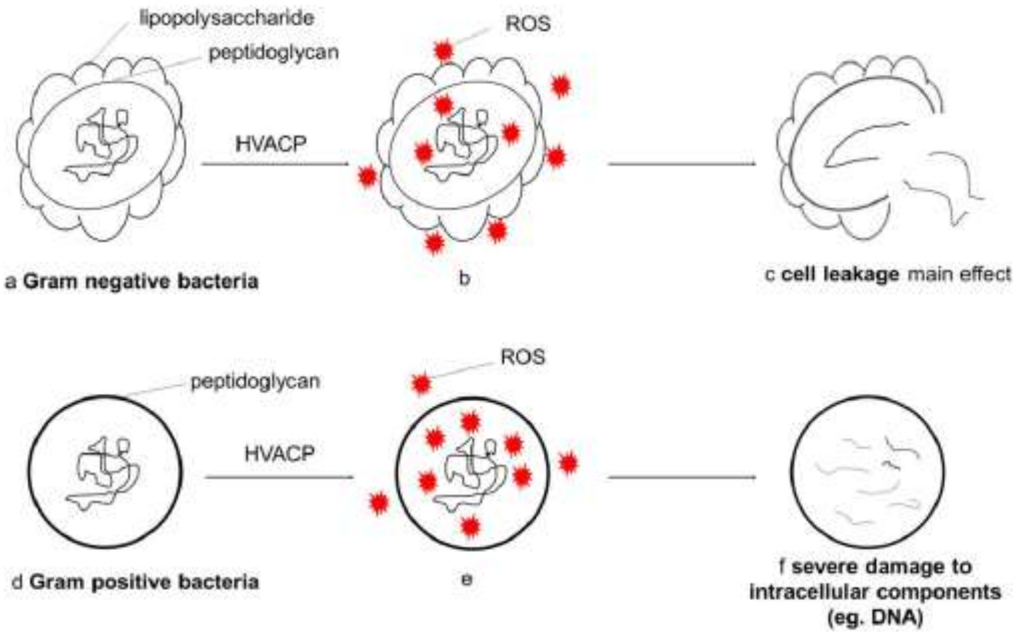


- Terminology: cold, cool and nonthermal
  - Cold, cool = operates at or near room temperature
  - Nonthermal = antimicrobial mode of action other than heat

Cold plasma

- Inputs to the system
  - energy (electricity, microwaves, etc.)
  - carrier gas: air, a pure gas (He, Ar, O_2, N_2, etc.) or a defined gas mixture
- Output
  - self-quenching plasma
  - resolves to UV light and ozone
  - chemical residues are expected to be minimal to non-existent
- Relatively new technology for food processing
  - adaptation from existing applications
  - regulatory status

Cold plasma mode(s) of action

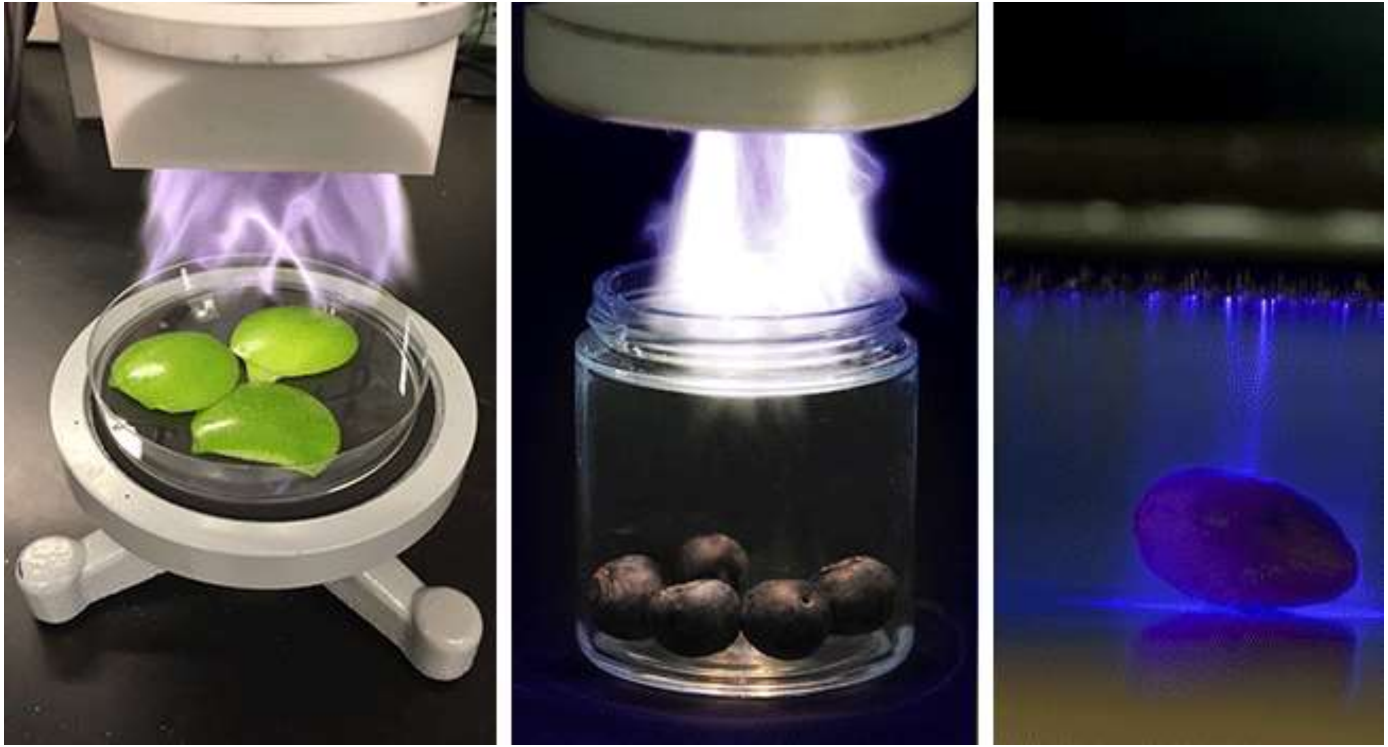


Mechanisms of Inactivation by High-Voltage Atmospheric Cold Plasma Differ for *Escherichia coli* and *Staphylococcus aureus*

L. Han,[a] S. Patil,[a] D. Boehm,[a] V. Milosavljević,[a] P. J. Cullen,[a,b] P. Bourke[a]

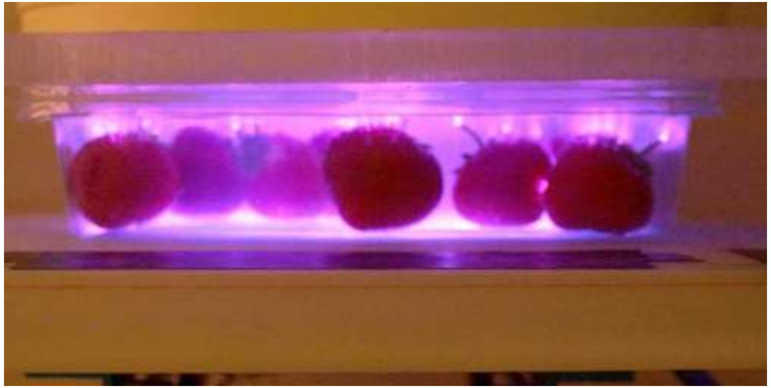
School of Food Science and Environmental Health, Dublin Institute of Technology, Dublin, Ireland[a]; School of Chemical Engineering, UNSW, Sydney, Australia[b]

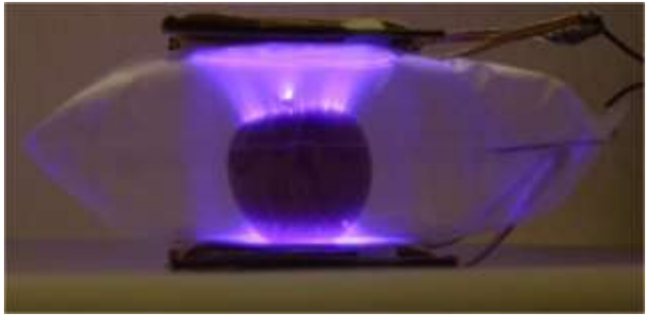
Flexible, broadly effective



Why *in-package* cold plasma?

- Terminal processing steps
- Heat, irradiation, some MAP
- Active packaging
  - Chlorine dioxide
  - Volatile oils
- Cold plasma as a potential in-package terminal processing step
- Potential for single or multiple treatments

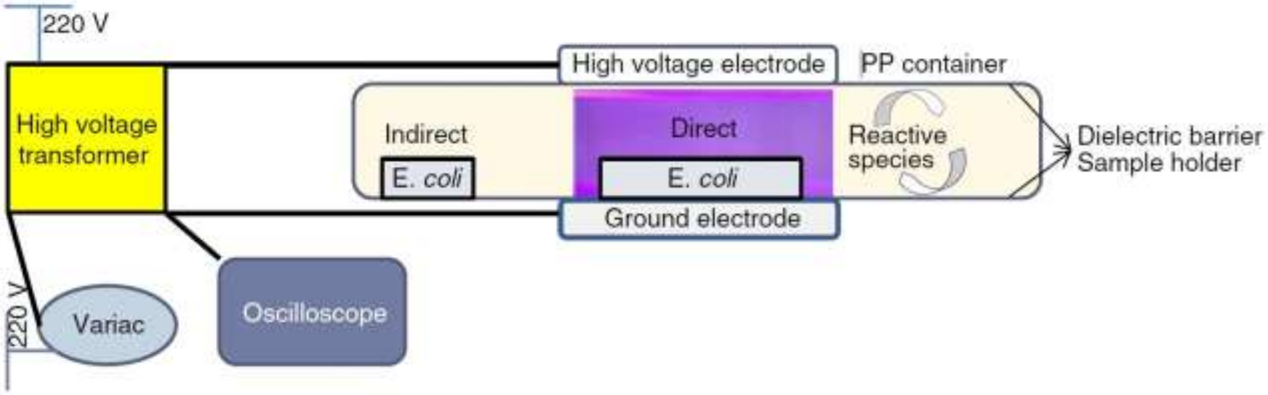




Overview of recent research

- Media-based studies and in vitro suspensions
- Produce studies: strawberries, lettuce, tomatoes, mixed salads
- Inactivation of pathogens
  - *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*
  - Spore formers
- Polymer modifications
- Pesticide residue inactivation

Media studies, *E. coli*



- *Escherichia coli* ATCC 25922
- 300s DBD exposure, 22mm gap
- Variable treatment times; post-treatment die-off

(Ziuzina et al., 2012, J Appl Micro)

Media studies, *E. coli*

- Direct exposure to DBD more effective than indirect.
- Longer time = greater kill. Evidence of sublethal injury.
- Ozone concentrations up to 5200 ppm.

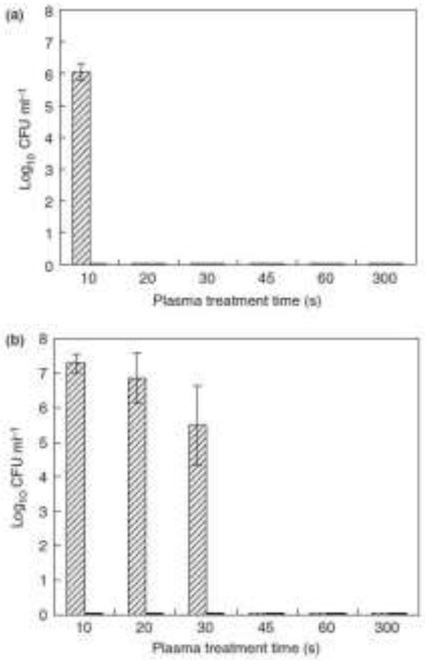


Figure 4 Effect of plasma treatment time (s) on inactivation efficiency of *Escherichia coli* ATCC 25922 (post-treatment storage time 24 h). (a) Direct plasma exposure: (■) phosphate buffered saline (PBS); (/) maximum recovery diluent (MRD). (b) Indirect plasma exposure: (■) PBS; (/) MRD.

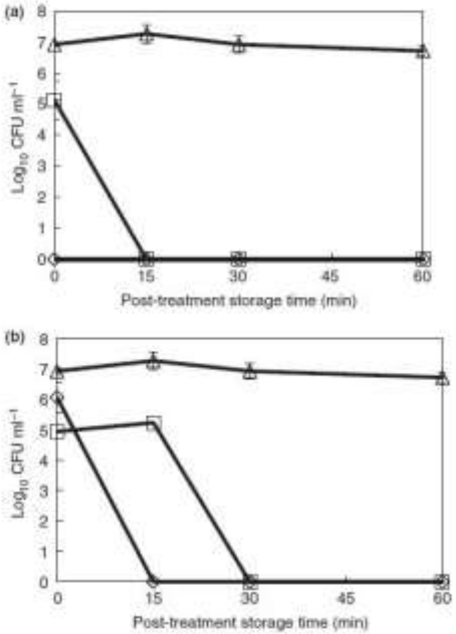
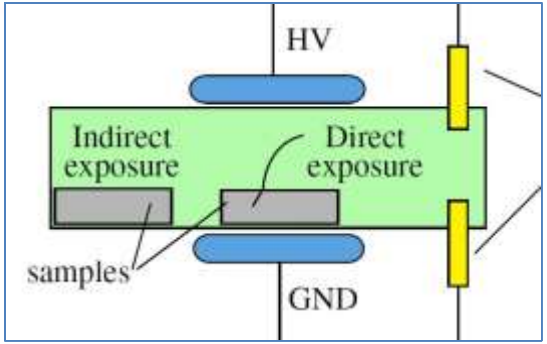
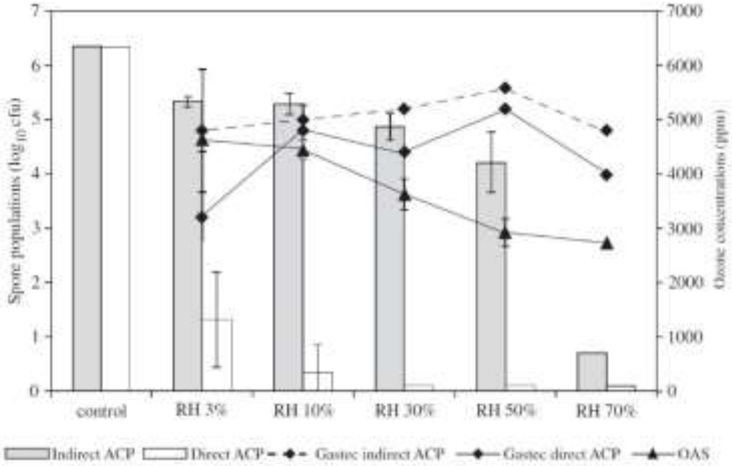


Figure 2 Effect of post-treatment (300 s) storage time on inactivation efficiency of *Escherichia coli* ATCC 25922. (a) Direct plasma exposure: (Δ) control; (◊) maximum recovery diluent (MRD); (□) phosphate buffered saline (PBS). (b) Indirect plasma exposure: (Δ) control; (◊) MRD; (□) PBS.

In vitro exposure, *Bacillus*





- *Bacillus atrophaeus* spores in petri dish; various gas mixtures & humidity levels; 60s treatments
- Up to 6 log reduction. Strongly dependent on strength of plasma field, and relative humidity
- Role of short-lived ROS and hydroxyl radicals derived from H_2O

(Patil et al., 2014, J Hosp Inf)

Strawberries, DBD

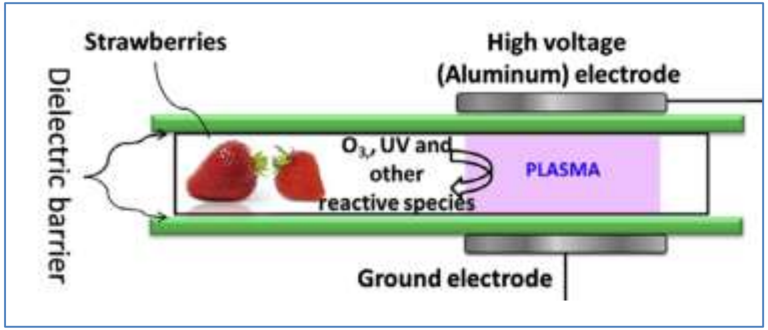


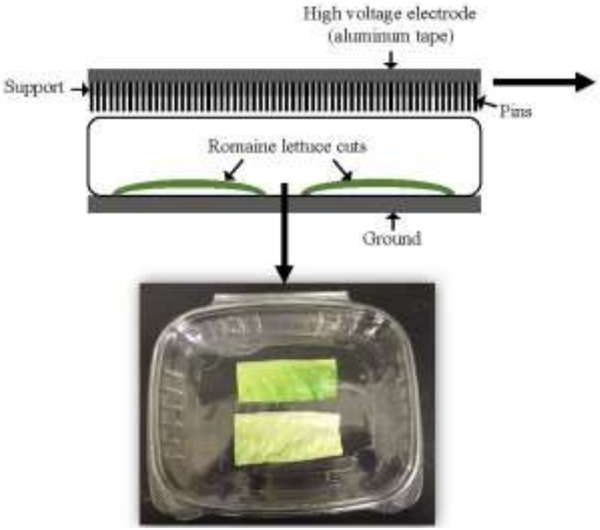
Table 2
Microbial reductions on strawberry surface by indirect atmospheric cold plasma (ACP) treatment in air.

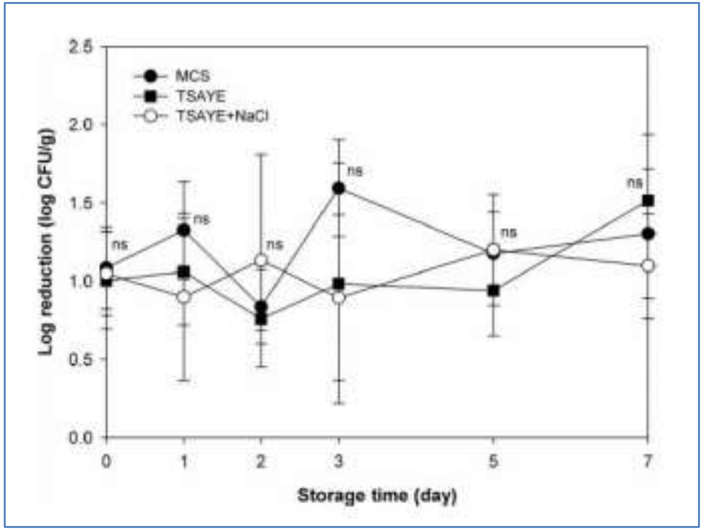
Microorganisms	Initial population (log_{10}cfu/g) untreated	Untreated control stored at 10 °C for 24 h	ACP treated surviving population (log_{10}cfu/g)
Total mesophiles	4.99 ± 0.02	4.92 ± 0.14	2.56 ± 1.82 (12–85% reduction)
Yeast/moulds	4.96 ± 0.08	5.06 ± 0.04	1.56 ± 1.29 (44–95% reduction)

- DBD in air for 5’, stored at 10C for 24h
- TAPC reduced by ~2.4 log; Y&M by 3.4 log
- Ozone concentrations of 1000 ppm
- No rise in temperature
- No significant effects on respiration, color, firmness

(Misra et al., 2014, J Food Engineering)

Romaine lettuce, *E. coli* O157:H7

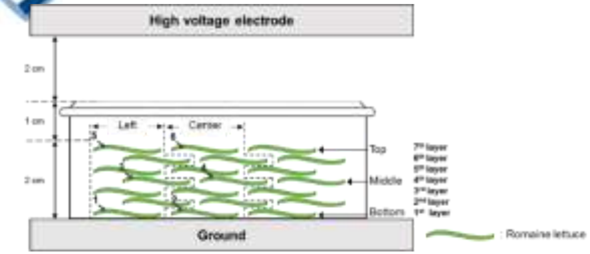




- Romaine lettuce, inoculated with E. coli O157:H7
- 5’ treatment, with plasma generated inside the commercial clamshell package
- 1.0 – 1.5 log reduction, persistent through 7d refrigerated storage

(Min et al. 2016, JFP; Min et al. 2016, Int J Food Micro)

Romaine lettuce: *L. mono.*



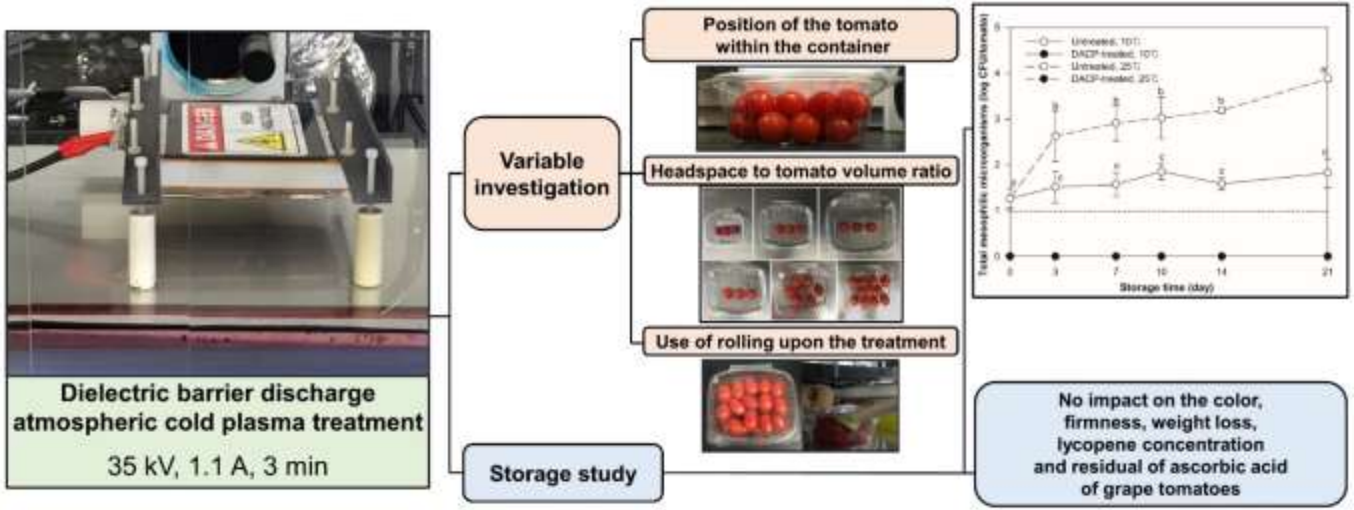
Schematic diagram of the cold plasma reactor holding a clamshell package treatment sample containing seven layers of lettuce leaves in a three-column configuration.

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

- Stacks of Romaine lettuce, inoculated with *L. monocytogenes.* Corona discharge plasma, 10’ treatment
- Cold plasma reduced L.m. throughout the stacks
- No effect on respiration, color, water loss

(Min et al. 2017, Food Micro)

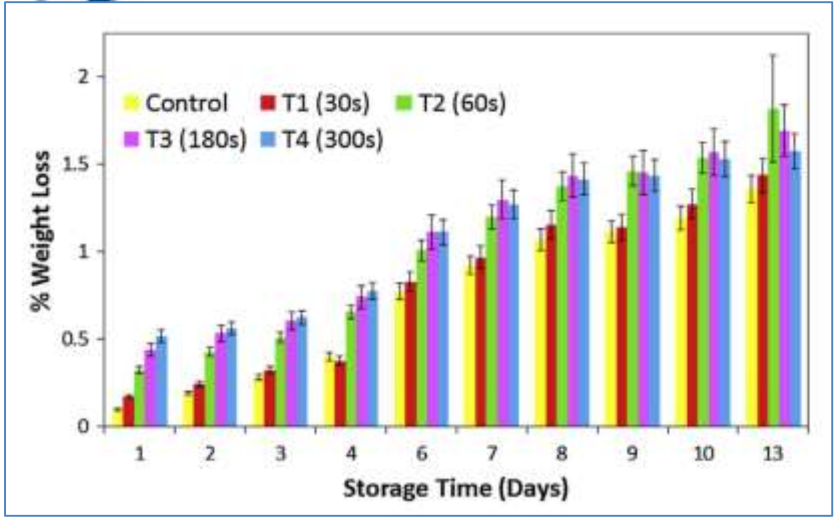
Tomatoes, *Salmonella*

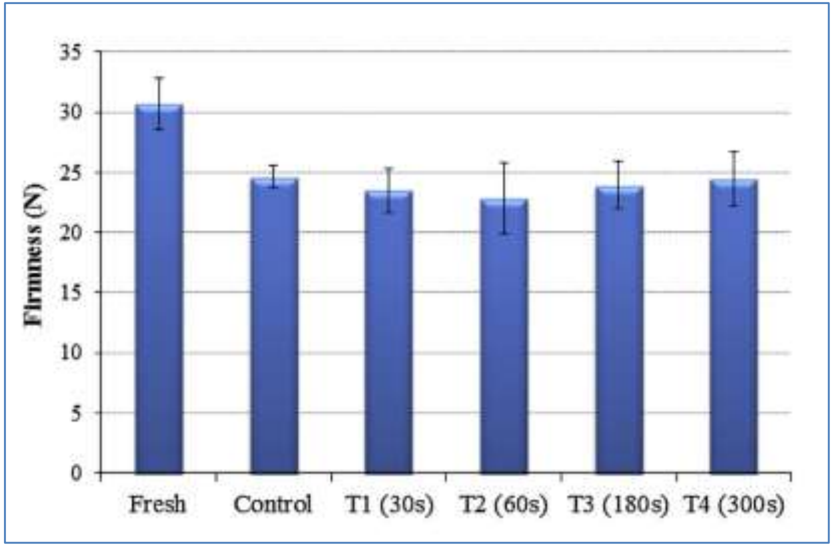


- *Salmonella* reduced by 3.3 ± 0.5 log CFU/tomato in stacked layers
- TAPC, Y&M reduced by 1.3 and 1.5 ± 0.2 log CFU/tomato
- Sensory qualities unaffected: no change in color, firmness, weight loss, lycopene, ascorbic acid during storage at 10 and 25 °C

(Min et al. 2018, Food Res Int)

Tomatoes, sensory properties

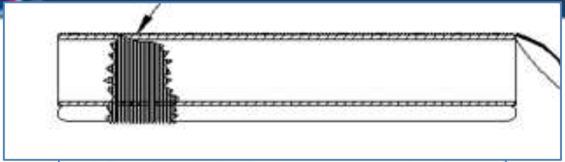


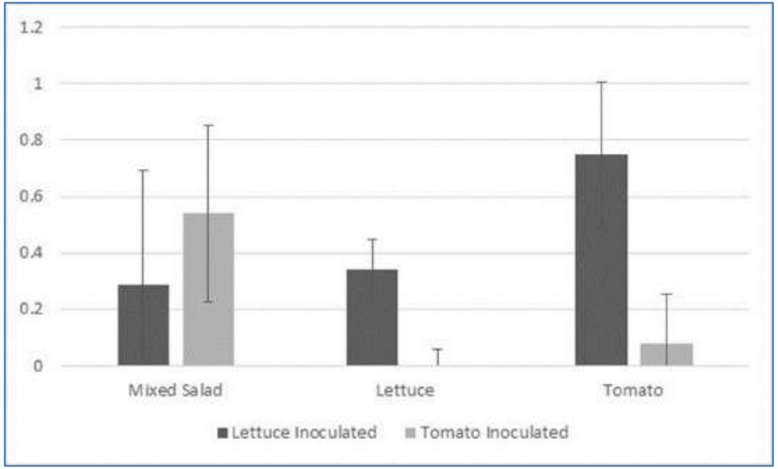


- Cherry tomato in air; indirect DBD for 30, 60, 180, 300s
- Variable respiration response based on treatment time
- Sensory qualities unaffected: no significant changes in pH, color, firmness

(Misra et al. 2014, J Biosci Bioengineering)

Mixed salads, *Salmonella*





- Tomato, lettuce, or tomato+lettuce in clamshell package; corona discharge, 3’.
- Contamination “direction” influenced efficacy of cold plasma
- More effective decontamination of mixed salad when Salmonella introduced via lettuce, but not when introduced via tomato

(Hertrich et al., 2017, JFP)

Cold plasma: polymers and pesticides

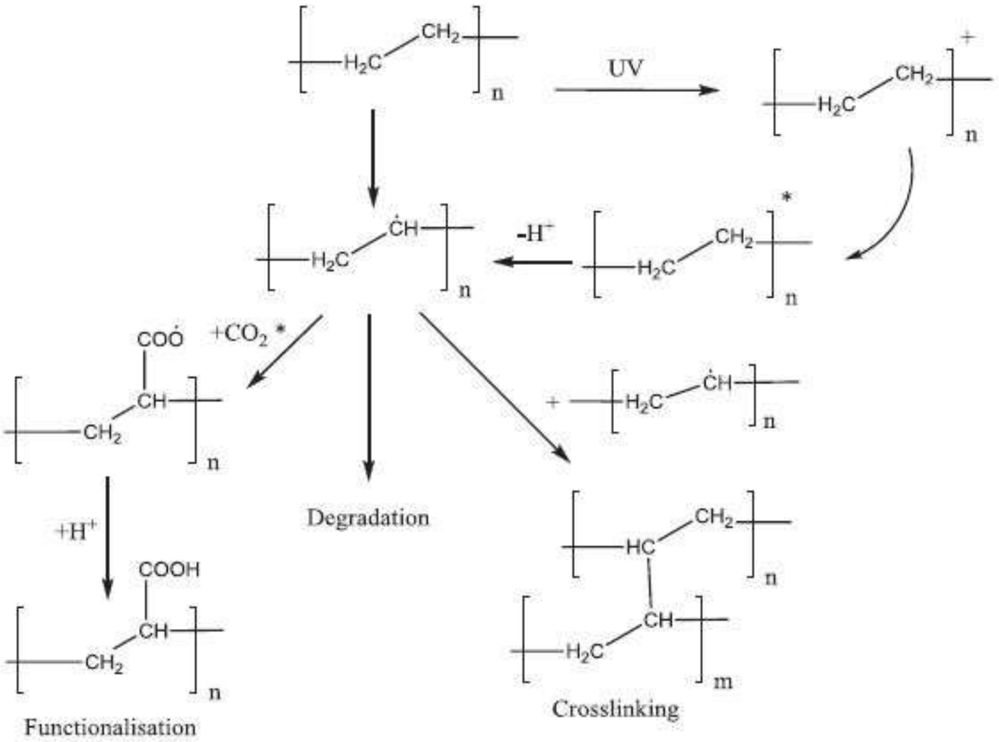
Cold plasma and polymers

- Surface treatments of packaging materials
- Functionalization: introduction of specific functional groups
  - wettability, sealability, printability, dye up-take
  - adhesion to other polymers
- Deposition
  - Labelling, adhesives, direct inking
  - Barrier properties, antimicrobial coatings
- Cleaning, etching

(Pankaj et al., 2014 Trends Food Sci Tech)

Cold plasma and polymers

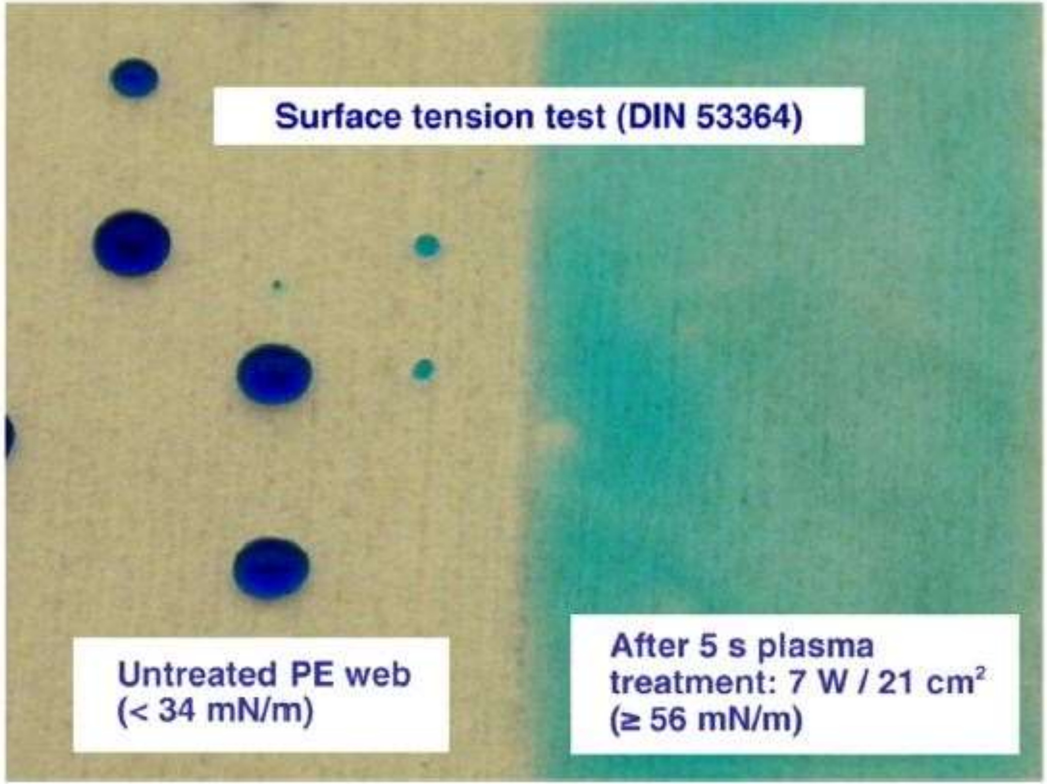
S.K. Pankaj et al. / Trends in Food Science & Technology 35 (2014) 5–17



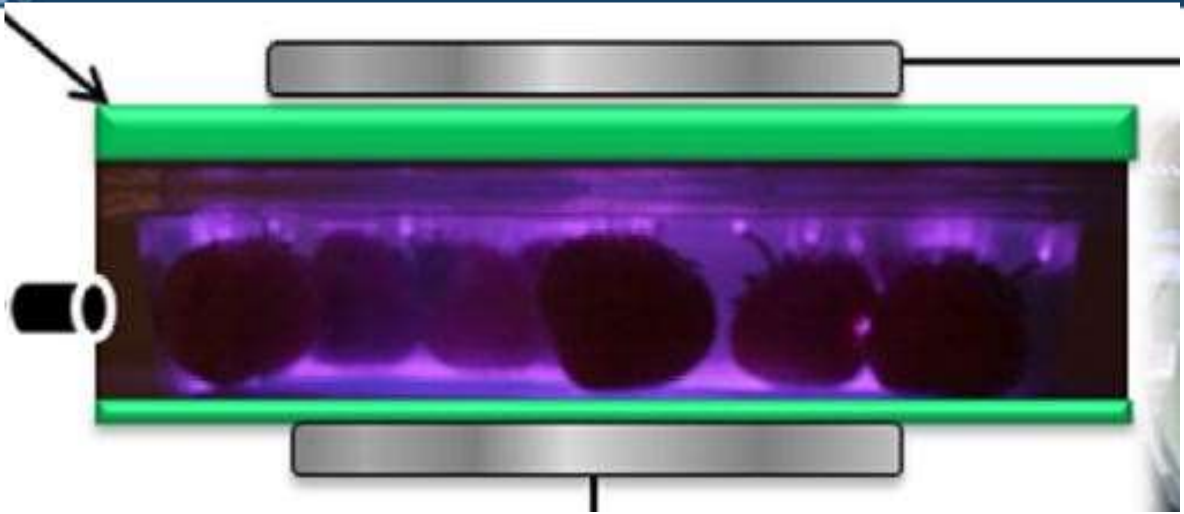
Cold plasma and polymers

- Ink applied to plasma-treated polyethelyene
- Change in surface energy alters interactions

(Bardos & Barankova, 2010, Thin Solid Films)



Pesticide degradation



- Azoxystrobin, cyprodinil, fludioxonil, pyriproxyfen, applied to strawberries
- Direct DBD treatment, 60, 70 or 80 kV
  - Higher voltage = greater plasma density

(Misra et al., 2014, J Haz Mat)

Pesticide degradation

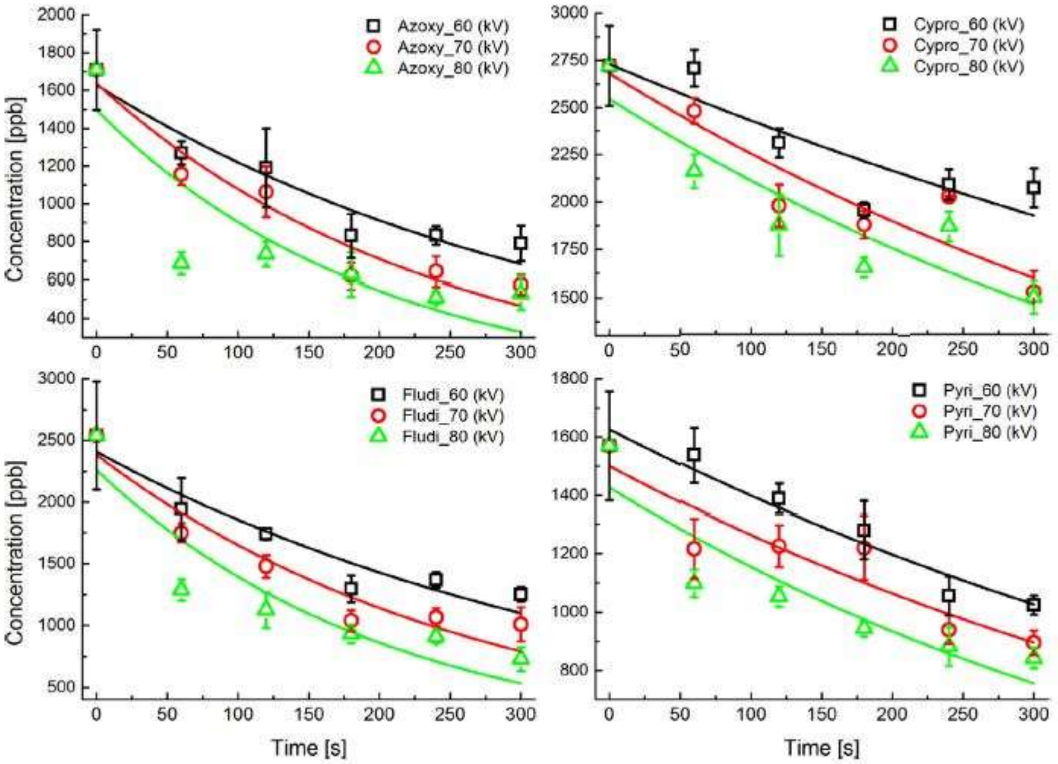
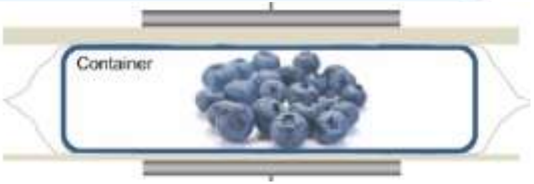


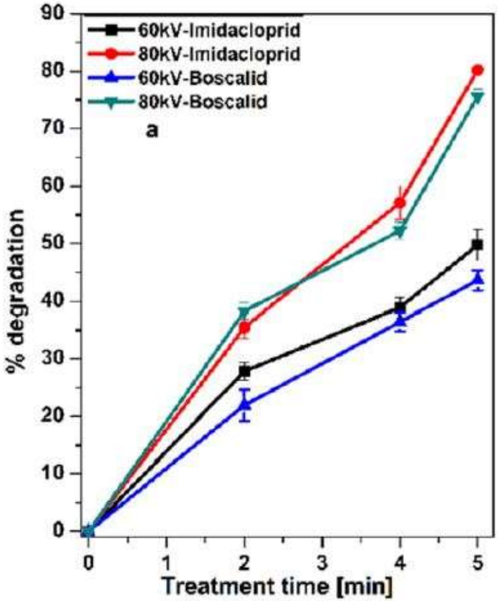
Fig. 5. Residual pesticide concentrations in strawberries before and after plasma treatment.

Pesticide degradation

- Pesticides boscalid and imidacloprid, applied to blueberries
- DBD, 80 kV, 5min
- Reductions of 80.2% for boscalid, 75.6% for imidacloprid
- Total phenol, flavonoid contents of blueberries increased
- Ascorbic acid decreased
- No significant effects on color, firmness.

(Sarangapani et al., 2017, Innovative Food Sci Emerg Tech)





Biological controls

Brendan A. Niemira
@Niemira

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Probiotics is rather like "The only thing that can stop a bad food with a bug is a good food with a bug."

4:22 PM - 2 May 2018

4 Retweets **15** Likes

Biological controls

- Competitive exclusion
  - Good bug occupies all the niches, so bad bugs can't take hold
- Inhibition
  - Good bugs stop the bad bugs from growing
- Predation
  - Good bugs actively kill the bad bugs

Competitive exclusion

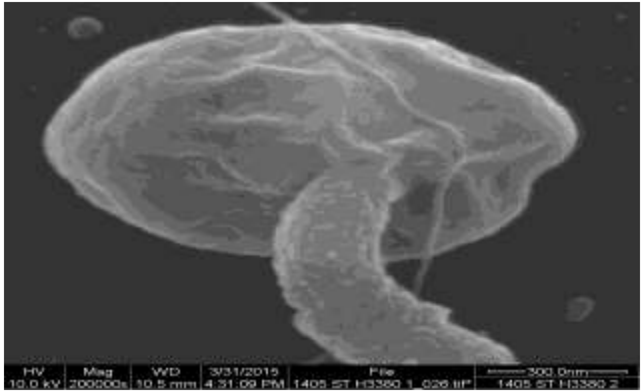
Efficacy of *P. fluorescens* on *E. coli* O157:H7 (*Ec*) inoculated on spinach (20 ºC)
(Dr. Modesto Olanya)

Treatment *P. fluorescens* (*Pf*) and *Ec*	**24 hrs** 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*) .

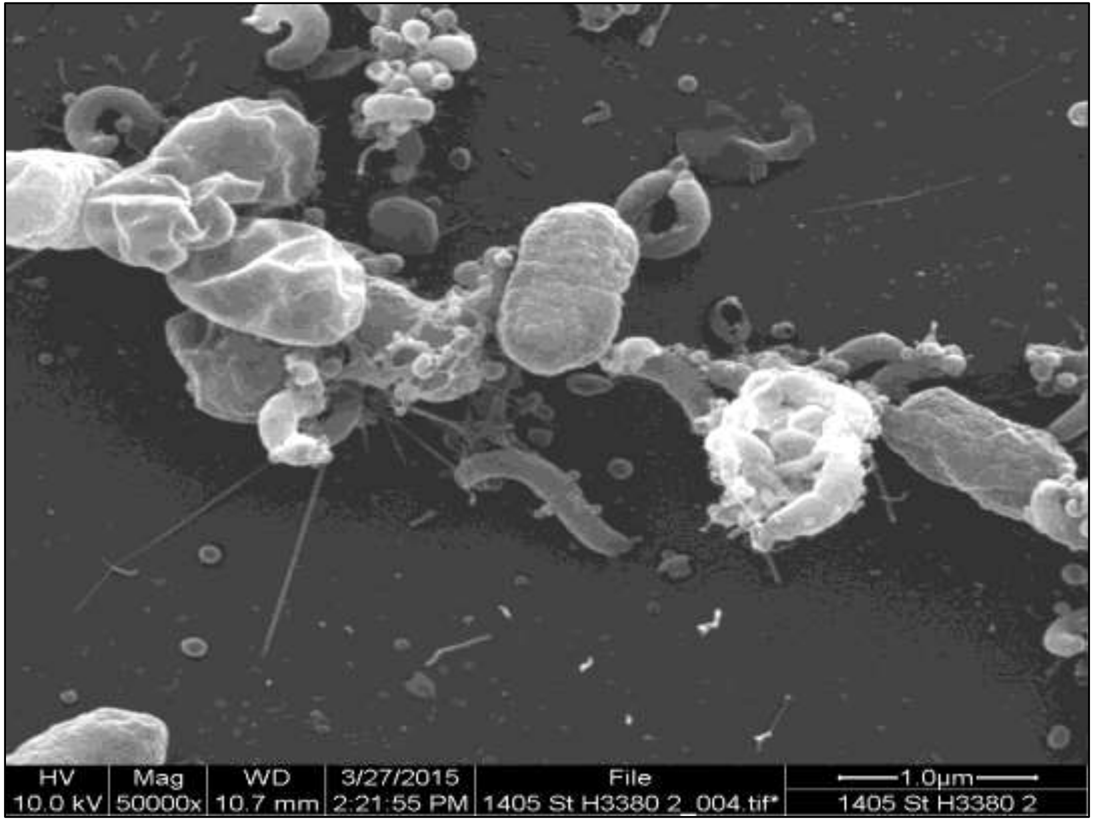
Predatory prokaryotes, bacteria predation

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



Predatory prokaryotes, bacteria predation

Predation of gram negative *Vibrio* by *Halobacteriavorax* (Dr. Gary Richards)

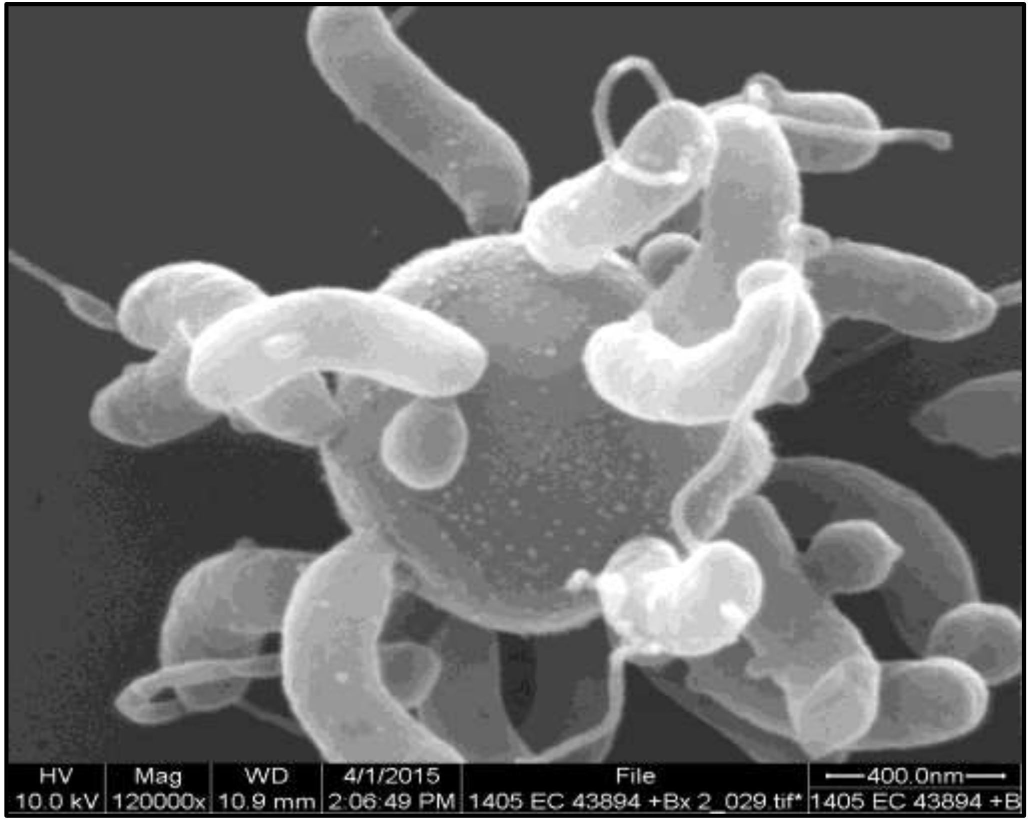


Predatory prokaryotes, bacteria predation

Swarming, membrane attachment, cell lysis

Effective against *Vibrio* spp.

Research underway to determine antagonism for *E. coli* O157:H7 and *Salmonella*



Summary

- Nonthermal interventions can effectively reduce pathogen loads
- Optimization for commodity, desired performance metrics
- Scale-up, commercialization

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