Human Pathogenic Bacteria Associated With Vegetables & Advances in Their Control Strategies

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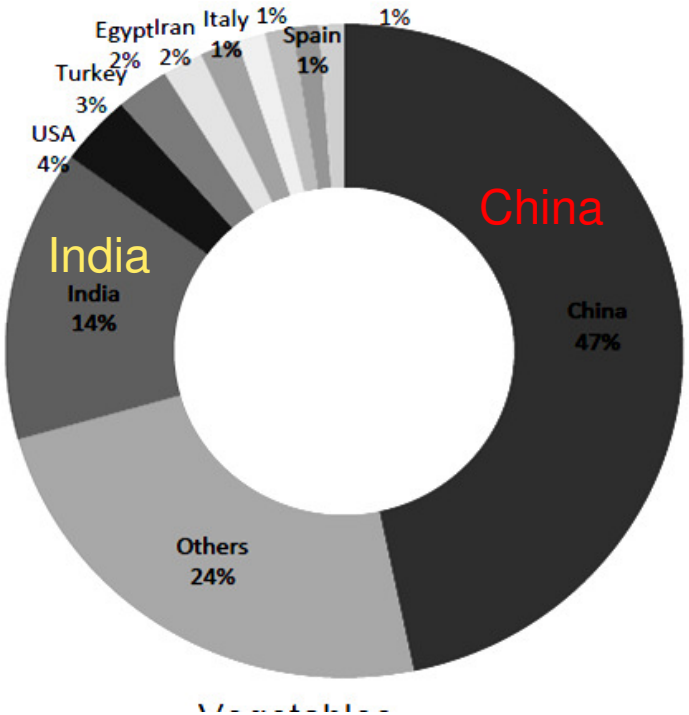
Treasure trove of nutrients

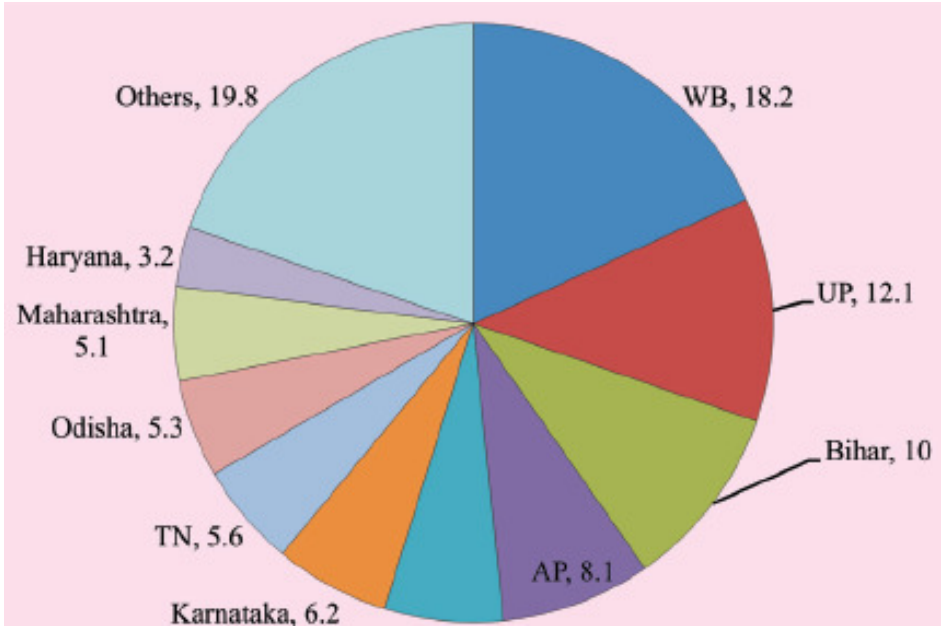
- $(CH_2O)_n$ - Most vegetables,
- Proteins – Legumes
- Antioxidants - Phenolics, Vitamins
- Phytochemicals - specific to the commodity
- Eg. Glucosinilates in cabbage, momordicin in bittergourd
- Dietary fibres : Most F&V
- Role in immunomodulation, possess functional properties – antidiabetic, anticancerous, prebiotic

"Integral part of diet world wide contributing taste and diversity "

Production scenario



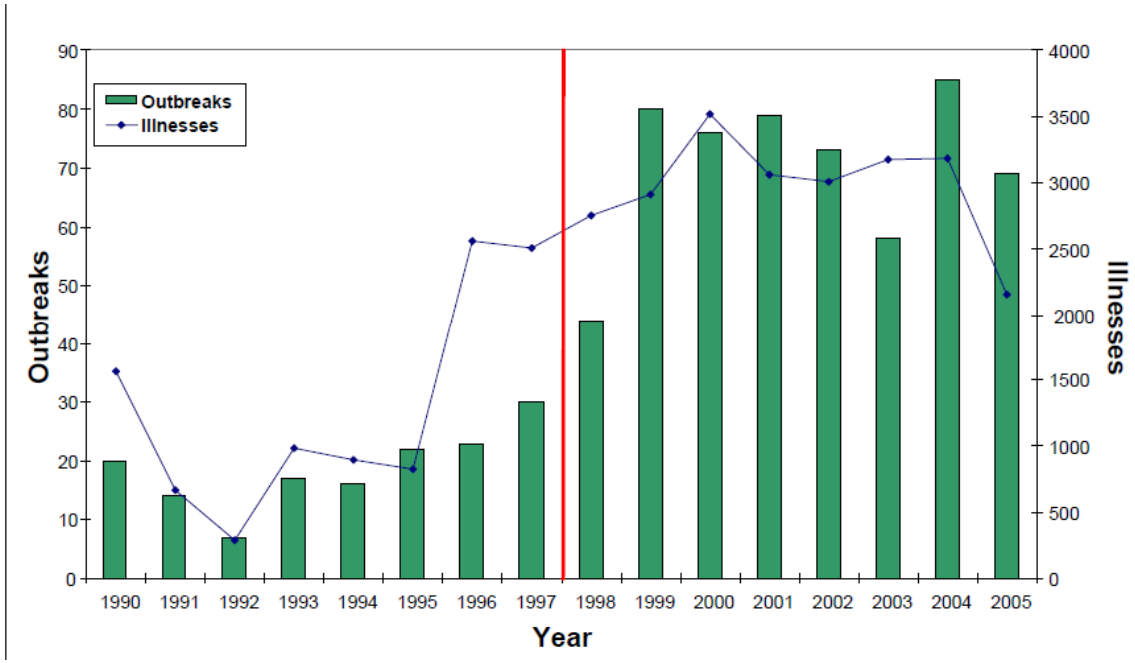
Source : FAO



Source : NHB, India

"Production and consumption forms a complex network; which makes quality control at consumer level difficult"

F&V consumption linked disease outbreaks



Source: CDC, USA

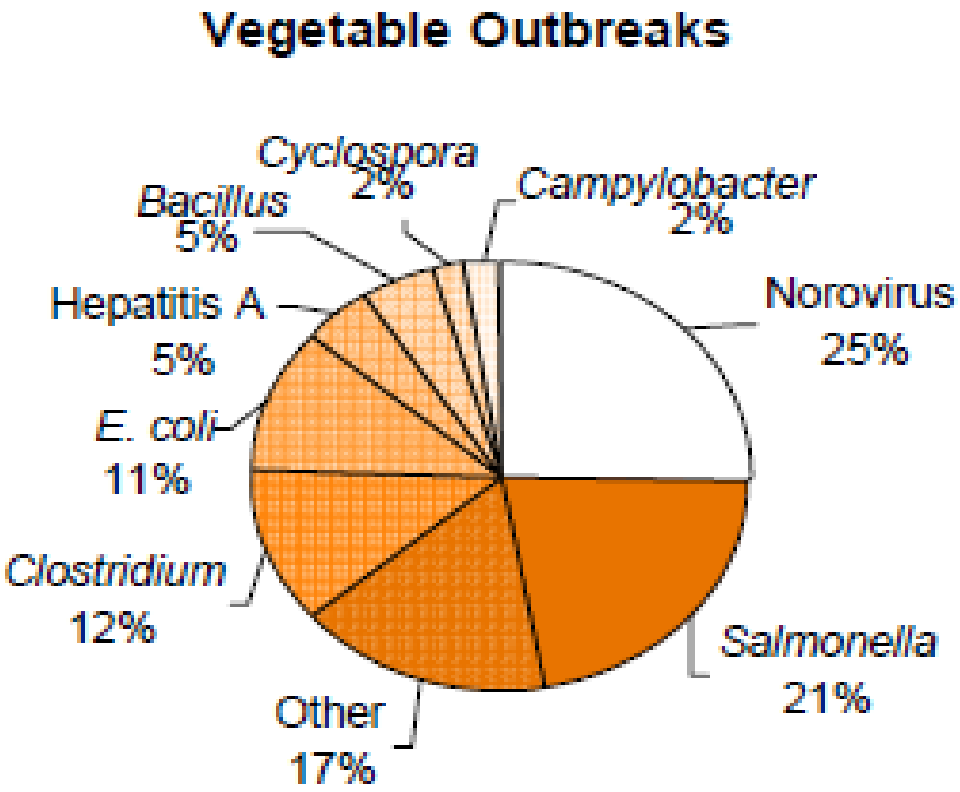
- 128,000 hospitalization and 3000 deaths each year

46% of all food borne illnesses 1998-2008 were attributable to fresh produce

Leafy green vegetables as the highest priority in terms of fresh produce safety from a global perspective. (WHO, 2008).

India lacks similar surveillance system; and the situation might be more precarious

Pathogens of concern

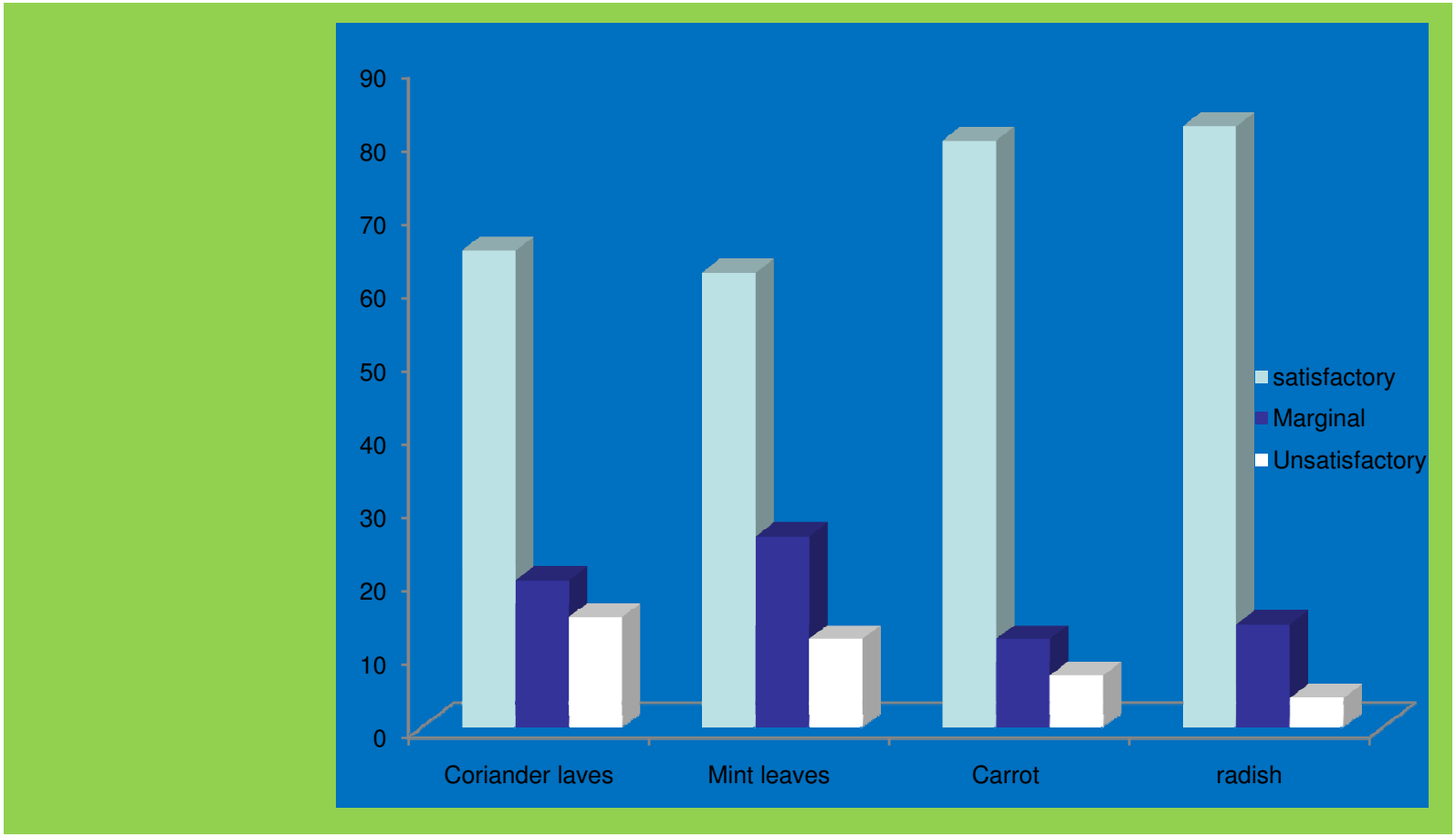


http://www.cspinet.org

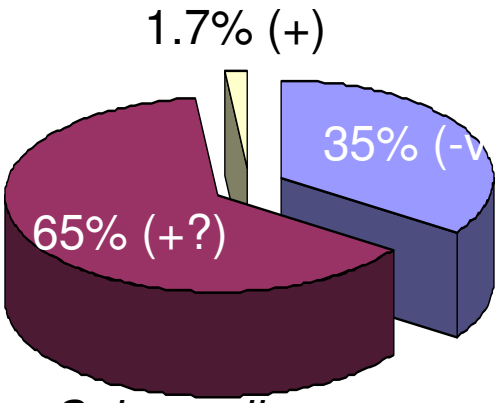
FSANZ guidelines for microbiological examination of ready to eat foods

Microorganisms	Microbiological quality			
	Satisfactory	Marginal	Unsatisfactory	Potentially hazardous
APC	N/A	N/A	N/A	N/A
E.coli/g(indicator organisms)	<3	3-100	>100	**
Salmonella sp. /25g	Not detected in 25g	-	-	Detected
Listeria monocytogenes	Not detected in 25g	Detected but $< 10^2$		$>10^2$
Bacillus cereus/g	$<10^2$	10^2-10^3	10^3 -10^4	$>10^4$

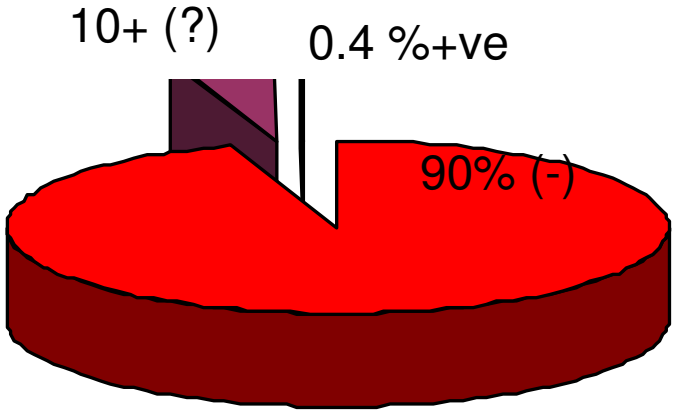
E coli population in Salad vegetables from Bangalore market



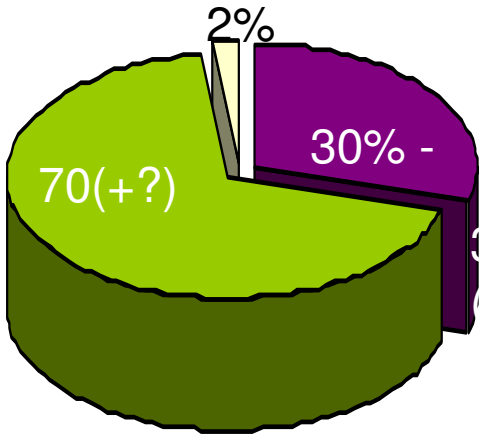
Distribution of food borne pathogens in vegetables from Bangalore market



Salmonella sp



Listeria monocytogenes



Yersinia enterocolitica

- **High no. of presumptive positives for enterobacteriaceae pathogens**
- **Could not detect *Bacillus cereus (n=75)***

Routes of Contamination

SOURCES

- Manure
- Soil
- Irrigation water
- Workers' hands
- Boxes

- Workers' hand
- Work benches
- Conveyer belts
- Washing water

- Workers' hands
- Cutting Board
- Cross-contamination meat
- Bad Storage

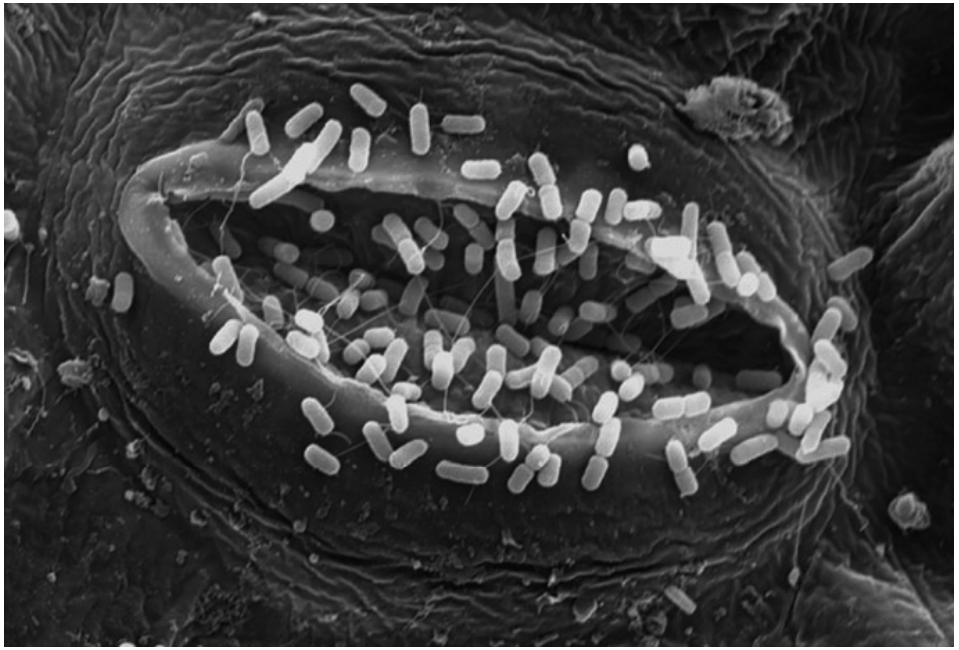
Contamination can happen at any point of production and handling

Routes of Contamination Contd..

- Seed as source of contamination in seed sprouts
- Conducive environment for the multiplication of pathogens

Do the human pathogens interact with plants : Cross kingdom pathogens?

- Salmonella and E coli gets internalized in the plant tissues- Chemotaxis
- Role of animal pathogenicity genes on the survival in plants is proven (Schikora et al., 2011)
- Immune suppression has been observed in Arabdiopsis infected with Salmonella
- A cross kingdom pathogen?



Internalization of E. coli in leaf stomata (Berger et al., 2010).

Survival of food borne pathogens in fresh-cut produce

- The studies using standard MTCC strains showed the growth of *Listeria monocytogenes* MTCC839 and the mere survival of *Salmonella enterica* MTCC 3219 in the minimally processed cabbage at low temperature storage (7^0C)

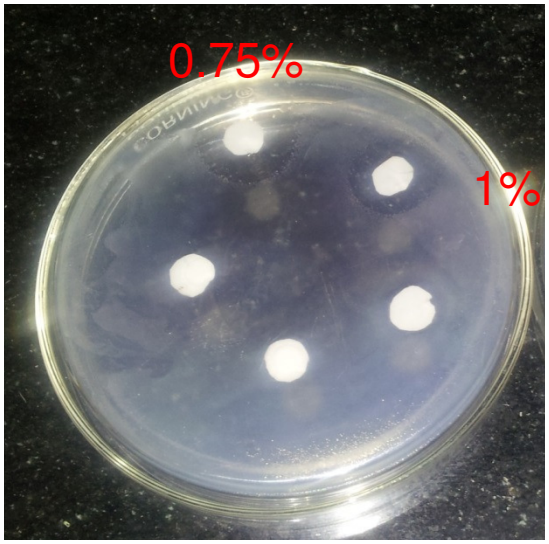
Control strategies

- **"Prevention is better than corrective measures"**
- Good agricultural practices
- Quality of Irrigation water is most important
- Properly composted manure: Temperature sh'd reach between 55 and 77 ^{0} C for 5 days
- Good Handling practices
- Washing is the critical control point
- Hygienic handling of the produce
- Proper storage

Sanitizing methods

Chlorine

- Most popular decontaminant
- Available as hypochlorite, chlorite
- Effective in 50-200ppm for >1 minute exposure
- Efficacy is <2 log reductions. So not useful in pathogen elimination
- Standard for testing newer methods
- Residual toxicity- So banned in fresh cut industry



In vitro inhibition of vegetable isolate of salmonella by chlorine

Sanitizing methods Contd.

Chlorine dioxide

- Max permitted concentration is 5ppm
- Results in 6 log reduction in bacterial count
- Tested against different pathogens in diverse F&V
- But requires treatment time 10minutes to 2hrs; Temperature >22 ^{0} C.

Peroxy acetic acid

- 40-80 ppm recommended by FDA
- Disinfection efficiency is similar to chlorine

Sanitizing methods Contd.

Ozone

- Disinfection capacity is equal to chlorine; but no residual effect.
- 5ppm is typically used
- Approved disinfection agent
- But corrosive, highly unstable, on site production is required
- Studies on using in packaging is under progress

Hydrogen peroxide

- No residue (GRAS), 0.5-2,5% is used
- Cause browning

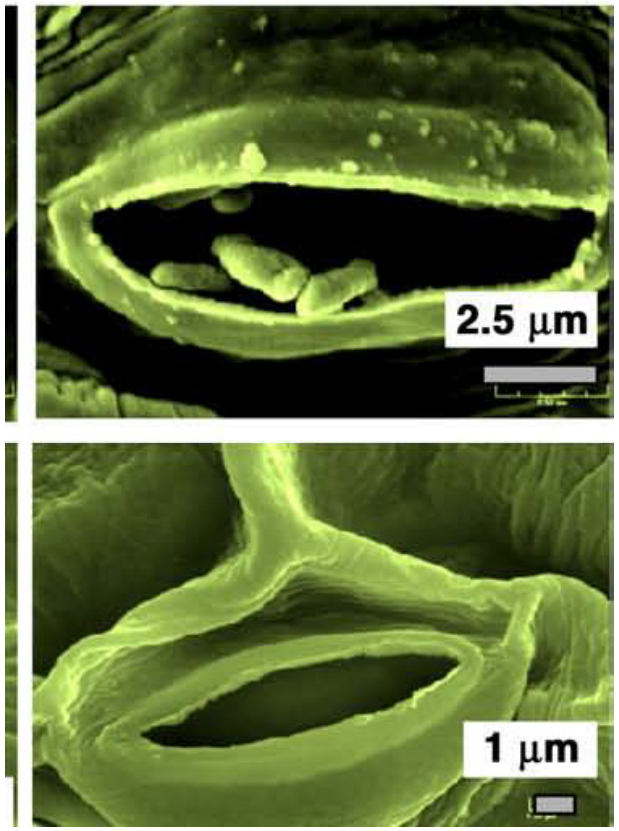
Efficacy of disinfectants in removing Salmonella from fresh-cut cucumber

Sanitizer	After 6 days (cfu/g) storage at 8^0 C	Marketability after 6 days
Control	$145X10^4$	2/5
100 ppm chlorine	$12X10^2$	3/5
2000ppm H_2O_2	104×10^3	2/5
Calcium propionate	37×10^2	4/5

population in the dip solution: $32X10^6$/ml in dip solution

Irradiation

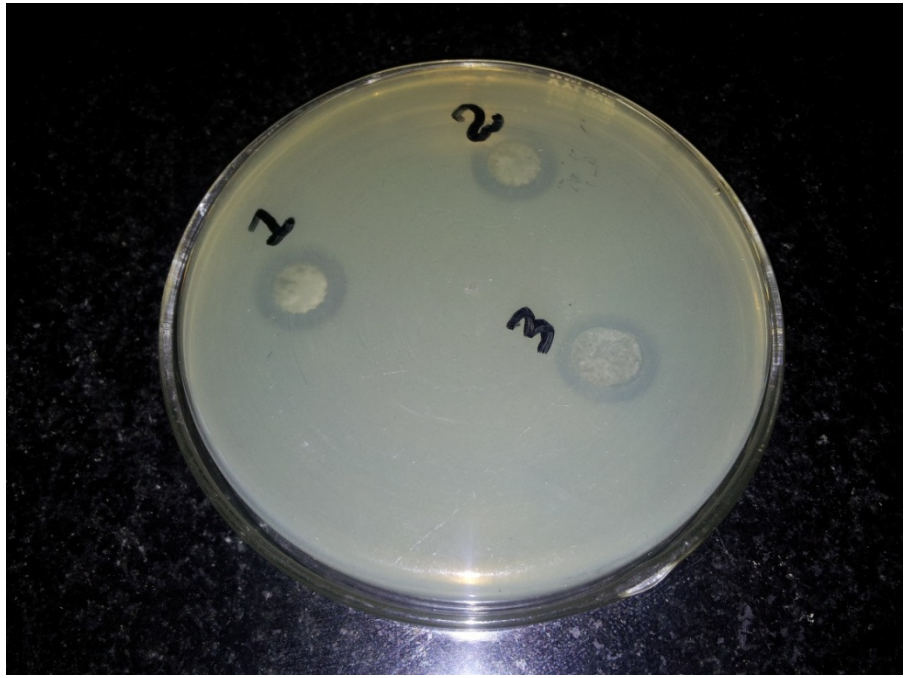
- Gamma irradiation approved in leafy vegetables for E. coli & Salmonella decontamination (FDA, 1999, 2008).
- Up to 10k Gy is permitted. > 1 kGY is likely to affect the shelf life
- Presence of oxygen improves the sensitivity of organisms
- Initial washing is necessary
- Requires high investment
- UV radiations (200-280nm) are also germicidal on the surface



(Gomes et al, 2009)

Bio-preservation

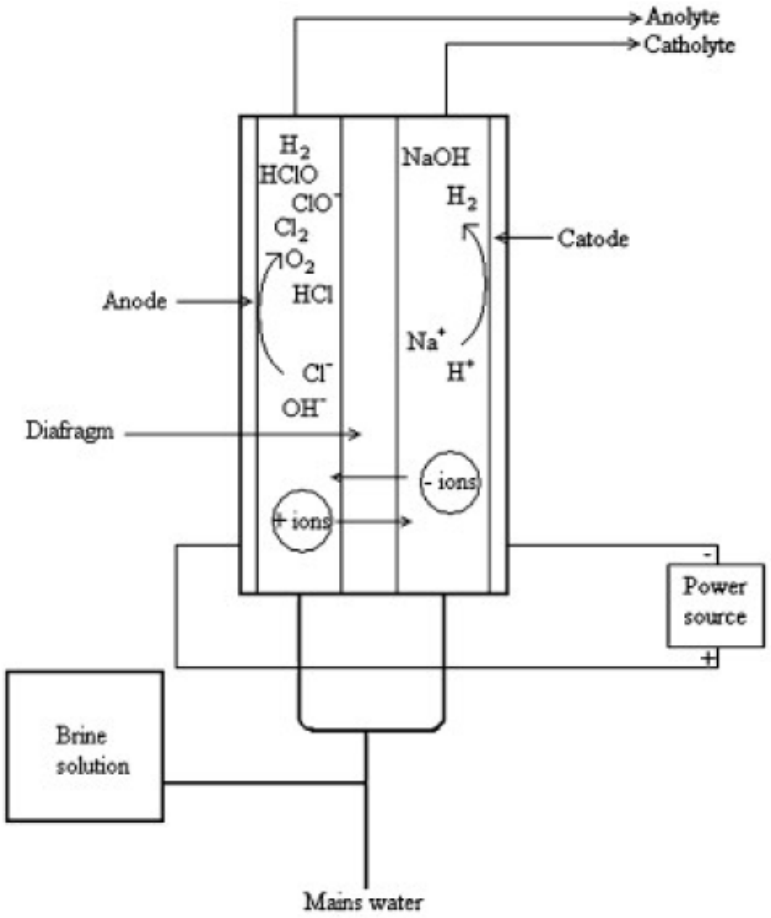
- ***Lactic acid bacteria:*** Through competition, production of antimicrobials like bacteriocins, H_2O_2



In vitro inhibition of salmonella cultures by lactic acid bacteria

Electrolyzed water

- Generated by the electrolysis of a diluted NaCl solution passing through the anode of a membrane electrolyzer.
- AEW has a strong bactericidal effect on most known pathogenic bacteria due to its low pH (2–4) and high oxidation–reduction potential and because it also contains active oxidizers like hypochlorous acid



Hsu, 2003)

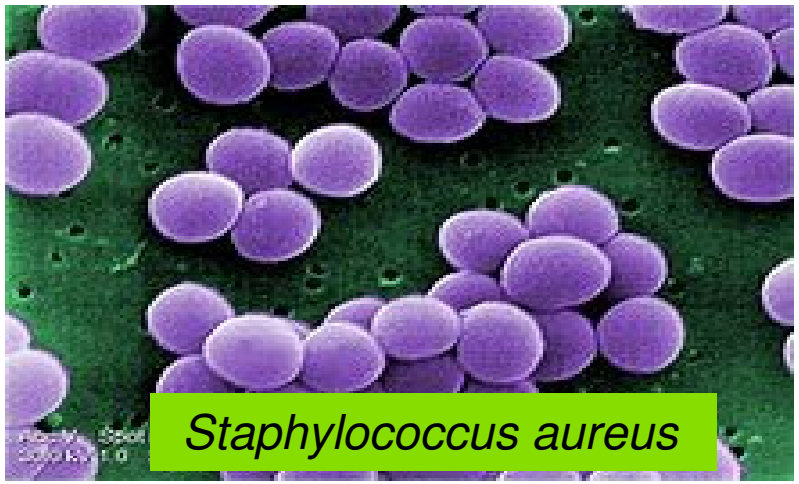
Other methods

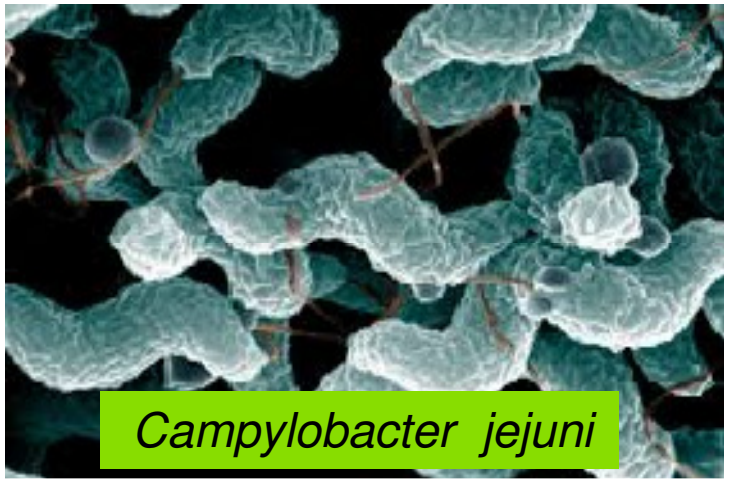
- Detergent solutions
- Organic acids
- Electrostatic applications of organic acids, Ultrasound, Pulsed light
- " Decontamination efficacy is variable with type of produce, adhering particles, biofilm formation & extent of tissue damage"
- "Every sanitization method has some drawbacks"

Thank You !

Other Food borne Bacterial Pathogens

- *Yersinia enterocolitica* :Grows at chilling temp
- *Shigella sonneii* & *S. flexnerii*: Diarrohea in children
- *Bacillus cereus*
- *Campylobacter :* diffuse, bloody, edematous, and exudative enterititis
- *Staphylococcus aureus*





Salmonella: Most frequent pathogen in F&V

- Family : enterobacteriaceae
- Salmonella enterica are human pathogenic
- S. enterica has 6 subspecies &>2500 serotypes
- Causes non-typhoidal salmonellosis
- Responsible for 76%, 60% and 30% of outbreaks caused by fruits, seed sprouts and leafy vegetables, respectively
- Self limiting diarrhoea, abdominal cramps, fever
- Infectious dose 10-10000 cells
- Subclinical levels of population always present in cattle gut

Pathogenic *E coli*

- **Shigatoxigenic E. coli** (STEC) is one of the pathotypes. It causes symptoms ranging from mild to severe and bloody diarrhoea.

- **Enterohaemorrhagic E. coli (EHEC):**subset of STEC typically associated with bloody diarrhoea and HUS, which produce cytotoxins, known as verotoxins (VT) or Shiga-like toxins (Stx).

- In relation to public health, **E. coli (O157:H7)** strain is the most important EHEC serotype linked to foodborne disease, resulting in a high incidence of EHEC infections and deaths each year.

- Microbiological criteria for fresh produce by several countries insists the absence of **E. coli (O157:H7**) in the sampling unit of food

Routes of Contamination Contd..

- Soil is natural habitat for many pathogens
- Eg. *Bacillus cereus, L. mono, Campylobacter*
- Animal manure adds to the load
- Eg. *Salmonella* and *E coli* population in feces ranges from 2-5 log cells
- Survival in solid manure : *Campylobacter* < *Listeria* < *Salmonella* < *Ecoli* O157:H7 (Nicholson et al., 2005)
- Moist clayey soil enhances their survival chances
- Spray and flood irrigation increases the contamination levels

Listeria monocytogenes

- G +ve, facultative anaerobes, motile at <30 ° C
- Identified as foodborne in 1981 in Canada, where listeriosis was linked to the contaminated cabbage consumption
- Estimated 1,600 illnesses and 260 deaths in the United States (U.S.) annually.
- Psychrotrophic
- Infectious dose <10 cells