UNIVERSITY OF MINNESOTA

Microbial Food Safety Issues of Organic Foods

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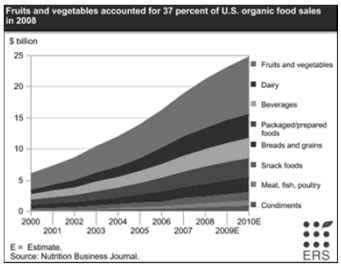
Definitions

- Organic foods

  Foods that are produced without the use of prohibited synthetic fertilizers and pesticides, sewer sludge, genetic engineering, growth hormones, irradiation and antibiotics; … applying natural pesticides and compost manure and using feeds grown in accordance with these practices for organic livestock

(USDA's Organic Rule: 7 CFR 205)

Organic Foods Market



3% total food sales in 2008

(http://www.ers.usda.gov/briefing/organic/demand.htm)

Organic Market Growth

- **Increased demand for:**
  - **Pesticide-free foods**
  - **"More natural" foods**
  - **Fresh foods**
  - **"More nutritious" foods**
  - **Ecologically/environmentally-friendly food**

Issues Related to Organic Foods

Safety of organic foods: a highly polarized issue

Safety Issues of Organic Foods

- **Positive aspects**
  - Lower pesticide levels
  - Less antibiotic-resistant bacteria
- **Negative aspects**
  - Heavy metals
  - Natural toxins/mycotoxins
  - **Pathogenic microorganisms**

Safety Issues of Organic Foods: Microbial Pathogens

- **Fresh fruits and vegetables**
  - Use of animal manure
  - Minimally processed ready-to-eat

- Meats
  - Antibiotic-free systems
- **Lack of organic disinfectants or sanitizers**

What is the risk of organic foods as vehicles of foodborne pathogens?

Pathogenic Microbes and Organic Foods

- Epidemiological evidence
- Presence of pathogens and indicators on foods
- Assessment of current practices
  - Use of manure
  - Limited number of approved sanitizers

Organic Produce Risk: Epidemiological Evidence

CDC's Food Outbreak Online Database (FOOD)

1998-2009 - >14,000 outbreaks recorded

No single reference to an organic product

http://wwwn.cdc.gov/foodborneoutbreaks

Organic Produce Risk: *E. coli* O157:H7 outbreaks or sporadic cases linked to manure

Manure type	Year/Place	# Cases	Vehicle	Reference
Cow manure	1985/ U.K.	49	Potatoes	Morgan et al., 1988
Cattle manure	1991/Mass.	23	Apple cider	Besser et al., 1993
Cow/calf manure	1992/Maine	5	Vegetables	Cieslak et al., 1993
Cattle carcass & manure	1993/Africa	>1,000	Water	Isaacson et al., 1993
Caw manure	1997/U. K.	8	Mud	Crampin et al., 1999
Sheep manure	1999/Scotland	6	Water	Licence et al., 2001
Cattle manure	2000/Ontario	1,346	Water	Health Canada, 2000
Cattle manure	2003/Germany	2	Soil	Grif et al., 2005
Cattle manure	2002/Minn.	1	Soil	Mukherjee et al., 2006
Cattle manure	2005/Sweden	135	Lettuce	Soderstrom et al., 2008

(Guan and Holley, 2003)

Organic Produce Risk: Epidemiological Evidence

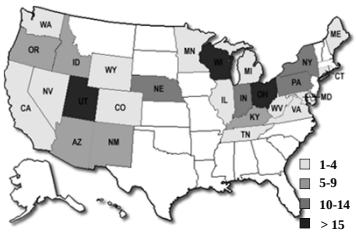
Outbreaks caused by "organic" produce

1995 – 42 cases of *E. coli* O157 in Montana due to lettuce
(Ackers et al, 1998)

1996 – 44 cases in CT & IL due to mesclun lettuce
(Hilborn et al, 1999)

1995 – *Citrobacter* outbreak in Germany linked to parsley
(Tschape et al, 1995)

Spinach Outbreak 2006



- 206 cases
- 103 hospitalizations
- 31 HUS cases
- 3 deaths

(CDC, 2006)

Spinach Outbreak 2006

- *Escherichia coli* O157:H7 found in
  - Wildlife
  - Spinach field
  - Environmental samples
  - Neighboring cattle farm (Paicines ranch)
- Mission Organics
  - Spinach farm was in its 2nd year transitioning to become certified organic
- Sign of the times or abnormality?

http://www.klubseniora.org/132

Organic Foods Risk: Epidemiological Evidence

Recent outbreaks caused by organic foods

- 2011 – Organic eggs in MN – *Salmonella* Enteritidis
  - 6 cases

2011 – Fenugreek sprouts in Germany – *E. coli* O104:H4

- More than 4,000 infections,
- >50 deaths

Pathogenic Microbes and Organic Foods

- Epidemiological evidence
- Presence of pathogens and indicators on foods
  - Recalls due to detection of pathogens
  - Survey prevalence studies
- Assessment of current practices
  - Use of manure
  - Limited number of approved sanitizers

Food Recalls Databases

http://www.fda.gov/Safety/Recalls/ArchiveRecalls/default.htm

http://www.fsis.usda.gov/Fsis_Recalls/Recall_Case_Archive/index.asp

Food Recalls Jan.-Nov. 2011

- Total food recalls (FDA + USDA) = 349
- Recalls caused by pathogens = 150
- Total organic food recalls = 6
  - Presence of allergens – 2 recalls
  - Presence of pathogens – 4 recalls (2.7%)
    1. Grape tomatoes – *Salmonella*
    2. Eggs – *Salmonella*
    3. Herbal tea – *Salmonella*
    4. Baby spinach – *Listeria monocytogenes*

Prevalence of Pathogens in Fresh Produce I

Vegetables tested	Total samples	Bacteria tested	% Positive samples	Place	Ref.
Imported	1,003	*Shigella* *Salmonella* *E. coli* O157	9 35 0	USA	FDA, 1999
Domestic	1,028	*Shigella* *Salmonella* *E. coli* O157	5 6 0	USA	FDA, 2000-01
Organic	3,200	*Salmonella* *E. coli* O157	0 0	UK	Sagoo et al., 2001
Bagged salad mixes	3,826	*Salmonella* *L. monocytogenes* *E. coli* O157 *Campylobacter*	6 1 0 0	UK	Sagoo et al, 2003
Organic and conventional	605	*Salmonella* *E. coli* O157	2 0	USA	Mukherjee et al, 2004
Organic lettuce	179	*Salmonella* *L. monocytogenes* *E. coli* O157	0 2 0	Norway	Loncarevic et al, 2005

Prevalence of Pathogens in Fresh Produce II

Vegetables tested	Total samples	Bacteria tested	% Positive samples	Place	Ref.
Organic and conventional	2,029	*Salmonella* *E. coli* O157	0 0	USA	Mukherjee et al, 2006b
Leafy greens, cantaloupe	398	*Salmonella* *L. monocytogenes* *E. coli* O157	3 0 0	USA	Johnston et al, 2005
Sprouts	200	*Salmonella* *L. monocytogenes* *E. coli* O157	14 0 3	USA	Samadpour et al, 2006
Mexican and domestic	466	*Shigella* *Salmonella* *L. monocytogenes* *E. coli* O157	0 0 3 0	So. USA	Johnston et al, 2006
Local produce	673	*E. coli* O157 *Campylobacter* *Salmonella*	0 0 0	Canada	Bohaychuck et al, 2009

Pathogen Prevalence in Poultry Organic vs. Conventional

Source	Bacteria tested	Outcome	Place	Ref.
Broiler farms	*Campylobacter*	O- 100%, C- 37% Significant difference	Denmark	Heuer et al. 2001
Retail chicken	*Campylobacter* *Salmonella*	O – 76%, C – 74% O – 61%, C – 44%	MD	Cui et al. 2005
Broiler farms	*Campylobacter*	O – 89%, C – 66% Significant difference	OH	Luangto-hgkum et al, 2006
Turkey farms	*Campylobacter*	O – 87%, C – 83% No fecal prevalence difference	OH	Luangto-hgkum et al, 2006
Broiler farms	*Campylobacter* *Salmonella*	No significant difference	Holland	Van Overbeke et al, 2006

(Fox et al, 2008)

Pathogenic Microbes and Organic Foods

- Epidemiological evidence
- Presence of pathogens and indicators on foods
- Assessment of current practices
  - Use of manure
  - Limited number of approved sanitizers

Impact of Organic Practices on Pathogen Survival

- If compost manure is used: 131 – 170°F for minimum 3 days (in-vessel) and 15 days (windrow)
- If not composted, manure must be used:
  1. At least 90 days before harvesting if the produce do not come in contact with soil
  1. At least 120 days before harvesting if the produce do not come in contact with soil

(NOP/USDA, 7 CFR 205)

Origin of the Organic Manure Handling Regulations

- NOP's framework in 1999:
  - Largely based on *E. coli* O157:H7
  - Recognized the lack of scientific data available
  - Consulted with M. Doyle (UGA), C. Hovde (U. Idaho) and A. Maule (CAMR, UK)
  - Based on the FDA's "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" report

(Eric Sideman, personal communication)

Scientific Background for Organic Manure Handling Regulations

References:

1. Bolton, D.J., C.M. Byrne, J.J. Sheridan, D.A. Mcdowell, and I.S. Blair 1999. The survival characteristics of a non-toxigenic strain of *Escherichia coli* O157:H7. J. Appl. Microbiol. 86:407-411
2. Kudva, I.T., K. Blanch and C. Hovde 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl. Environ. Microbiol. 64:3166-3174
3. Maule, A. 1995. Survival of the verotoxin strain of *E. coli* O157:H7 in Laboratory-Scale Microcosms. In Coliforms and *E. coli*: Problem or Solution? Ed. Kayand, D. and Fricker, C. pp61-65. Gateshead, UK: Athenaeum Press Ltd
4. Wang, G., W. Zhao, and M. P. Doyle 1996. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. Appl. Environ. Microbiol. 62:2567-2570

Manure Composting

Types of composting:

1. In vessel
   - 131 – 170°F (55 - 77°C) for 3 days
2. Windrow
   - 131 – 170°F for 15 days
   - Turning materials 5 times

National Organic Program, 7 CFR 205.2

Limitations of Composting in Windrows

- Uneven temperature distribution
- Cross-contamination
- Diversity of composting systems

Impact of Organic Practices on Pathogen Survival

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(NOP/USDA, 7 CFR 205)

Pathogen Survival in Manure

Manure and conditions	Bacteria	Maximum survival (days)	Other findings	Reference
Cattle slurries at RT	*Campylobacter* *Salmonella* *E. coli O157*	90	Inactivated after 30 days at 55ºC	Nicholson et al 2005
Chicken manure @ 4°, 22° and 37ºC	*E. coli* O157 *S.* Typhimurium	261	Maximum DRT = 150 days	Himathongkham et al 2000
Cattle manure and slurries @ 4°, 22° and 37ºC	*E. coli* O157 *S.* Typhimurium	100 @ 4ºC	6-log reduction after 38 and 48 days	Himathongkham et al 1999a
Chicken manure at 20ºC	*S.* Typhimurium	100 @ a_w =0.07	6-log reduction @ a_w = 1 after 22 days	Himathongkham et al 1999b
Cattle manure	*S.* Dublin *S.* Senftenberg *S.* Typhimurium	183 204 204	Inactivated in composted manure after 14 days	Forshell and Ekesbo 1993

Pathogen Survival in Soil I

Soil, manure type and conditions	Organism	Maximum survival (days)	Other findings	Reference
Composted dairy and poultry manure	*E. coli* O157	154 (lettuce) 214 (parsley)	4-log reduction after 42 days	Islam et al 2004a
Sandy loam soil, dairy cattle manure	*E. coli* O157	> 84 (carrot)	3-log red. w/onions @ 64 days 2.3-log red. w/carrots	Islam et al 2004b
Sandy loam soil, cattle & poultry manure	*E. coli* O157 *Salmonella* *Campylobacter* *Listeria monocytogenes*	>32, <62 >62 >18, <32 >62	2-log reduction after 62 days	Hutchinson et al 2004
Fallow soil & silt loam soil, cattle manure	*E. coli* O157	41 (fallow) 92 (silt loam) 500 (frozen)	Clay increased persistance	Gagliardi and Karns 2002

Pathogen Survival in Soil II

Soil, manure type and conditions	Organism	Maximum survival (days)	Other findings	Reference
Sandy, clay and loam soils	*E. coli* O157	56 (sandy) 175 (clay & loam)	3-log reduction after 136 days	Fenton et al 2000
Cattle manure @ 25ºC	*E. coli* O157	56		Mubiru et al 2000
Manure-ammended soil	*S.* Typhimurium	63 (5ºC) 42 (22ºC)		Zibilske and Weber 1978
Silty clay loam & channery silt loam	*Cryptosporidium parvum*	120 (90%) 164 (99%)	High variability in survival	Kato et al 2004
Dry and wet soils	*Cryptosporidium parvum*	50 (-10 ºC)	Freeze-thaw cycles had little effect	Kato et al 2002

Pathogenic Microbes and Organic Foods

- Epidemiological evidence
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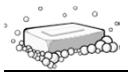


Sanitation

- Steps:

1. Cleaning: removal of soil from surfaces

2. Sanitizing: removal or killing of microorganism

Sanitizing

- Sanitizing methods

1. Physical
   - Heat: hot water and steam
   - Irradiation: UV light

2. Chemical

Sanitizing

- Chemical sanitizers

➔ No-rinse food contact surface sanitizer
  ➔ Reduces *E. coli* and *S. aureus* by 5 logs in 30 sec at 25°C

FDA Approved Non-Rinse Food Contact Surface Sanitizers

- Chlorine
- Chlorine dioxide
- Iodophors
- Quaternary ammonium compounds
- Acid-anionic sanitizers
- Carboxylic acid sanitizers
- Hydrogen peroxide
- Peroxy acid compounds
- Phenolic compounds

National List of Allowed and Prohibited Substances

- Includes non-synthetic and synthetic substances
- Three major categories:
  1. Livestock
  2. Crop production
  3. Processing

(USDA's Organic Rule: 7 CFR 205)

The National Organic List

- Criteria for synthetic substances:
  1. Consistent with organic production
  2. Cannot be produced from natural sources and there are no organic substitutes
  3. Does not have adverse impact on the environment
  4. Does not affect nutritional quality
  5. Is not a preservative or is used to improve quality after processing
  6. Is a GRAS substance
  7. Essential for handling organically produced products

(USDA's Organic Rule: 7 CFR 205)

Sanitizers in the National Organic List

Category	Livestock	Crops	Process
Alcohols (ethanol, isopropanol)			
Chlorine compounds (4 mg/L residual Cl) ▪ Sodium/calcium hypochlorite ▪ Chlorine dioxide			
Hydrogen peroxide			
Peracetic/peroxyacetic acid			
Iodophore			
Citrus product			
D-limonene			

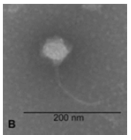
(USDA's Organic Rule: 7 CFR 205)

Alternative Sanitizers / Antimicrobial Ingredients

- Natural salts
- Natural plant extracts
- Microorganisms
  - Bacteria
  - Bacteriophages

Bacteriophages as Potential Antimicrobials for Organic Food Production

- Specific to host
- Obligate parasites
- Widely available in nature
- Cost-effective



Source: Dr. Andrew Brabban

- Other phages are approved for organic production
- Recent approval by FDA

Bacteriophages for Organic Foods

- Plant pathogen phages
  - *Xanthomonas campestris* /*Pseudomonas syringae* phages
    - AgriPhage® by Omnilytix, Inc.
- Human pathogen phages
  - *Listeria* phages (approved by OMRI as Microorganisms)
    - Listex P100 ® by Micreos, Inc.
    - ListShield® by Intralytix, Inc.

Bacteriophages as Potential Antimicrobials for Organic Food Production

Project goal:

- Isolate, screen and evaluate bacteriophages against:
  - *Escherichia coli* O157:H7 and other shiga-toxin producing *E. coli*
  - *Salmonella*
  - *Listeria monocytogenes*

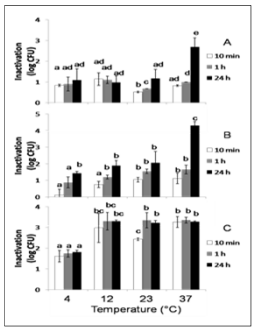
Phages vs. *E. coli* Range of Sensitive Strains

Bacterial species/serovars (number)	% Strains affected by individual phages							
	38	39	41	AR1	42	CEV2	ECB7	ECA1
O157:H7 (N=130)	96.2	93.1	97.7	93.1	99.2	96.0	93.1	93.1
O26 (N=10)	70.0	70.0	90.0	70.0	80.0	80.0	90.0	100.0
O111 (N=10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Commensal *E. coli* (N=19)	26.1	31.6	15.8	15.8	31.6	21.1	21.1	5.3
***E. coli* O55 (N=4)**	25.0	0.0	0.0	75.0	25.0	50.0	50.0	25.0
***Salmonella* (N=27)**	22.2	11.1	7.4	7.4	18.5	0.0	48.2	7.4

(Viazis et al. 2011a)

Use of Phage Mixture (BEC8) for treating dried *E. coli* O157 strains on stainless steel

MOI= PFU/CFU



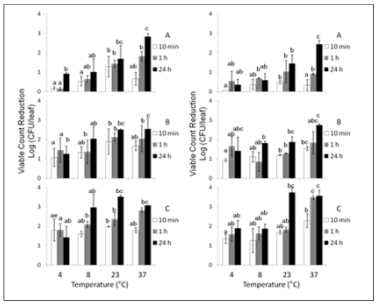
A: 1 MOI

B: 10 MOI

C: 100 MOI

(Viazis et al, 2011b)

Use of Phage Mixture (BEC8) for treating dried *E. coli* O157 strains on spinach and lettuce



Spinach Lettuce

A: 1 MOI

B: 10 MOI

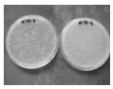
C: 100 MOI

MOI= PFU/CFU

(Viazis et al, 2011c)

Phages vs. *Salmonella* Efficiency of Plaquing (EOP)

Salmonella Strains	Plaque formation				
	SEA1	SEA2	SSA1	SSA2	STE1
S. Typhimurium I598	++	+++	+++	+	-
S. Typhimurium I527	+++	+++	+++	+	-
S. Typhimurium I534	+++	+++	++	++	-
S. Typhimurium I535	+++	+++	+	+	-
S. Typhimurium I526	+	+++	+++	+	-
S. Typhimurium E2009005811	+	++	+	-	-
S. Typhimurium 14028	+++	+++	+	-	++
S. Typhimurium I536	-	++	+++	+	Host
S. Typhimurium DT104	-	+++	-	-	-
S. Typhimurium UK-1	-	++	+	+++	-
S. Saintpaul E2008001236	++	++	Host	Host	-
S. Tenessee E2007005O2	+++	+++	+	-	++
S. Enteritidis 95657613	+	+++	+	+++	+
S. Enteritidis 2009595	Host	Host	+	+	-
S. Newport AMO7076	+++	+++	+	-	-
S. Newport AMO7073	+++	+++	-	+	-
S.. Newport B4442CDC	-	++	-	+	++
S. Montevideo 95573473	-	-	-	+++	-
S. Agona FDA	-	++	-	+	-



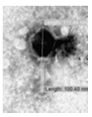
+++, EOP 1 to 0.5; ++, EOP 0.5 to 0.2; +, EOP 0.2 to 0.001

*EOP = phage concentration$_{testing\ strain}$/phage concentration$_{host\ strain}$

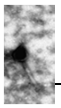
Phages vs. *Salmonella* Range of Lyzed Serovars

Phage	Host serovar	Cross infected Serovar
SSA1, SSA2	*S.* Saintpaul	Typhimurium, Enteritidis, Newport
STD2	*S.* Typhimurium	Enteritidis
STE1	*S.* Typhimurium	Newport
SEA1, SEA2	*S.* Enteritidis	Typhimurium, Newport, Tennessee

Phage	S. Typhimurium (N = 14) %	S. Enteritidis (N = 3) %	S. Newport (N = 6) %
SEA1	**64.3**	**100**	**66**
SEA2	**64.3**	**66.7**	**66**
SSA1	**78.6**	**100**	**0**
SSA2	**78.6**	**100**	**0**



SEA1



Phages vs. *Listeria monocytogenes* strains

Listeria monocytogenes Strains	Phage Isolates (EOP) Efficiency of plaquing									
	LMB3	LMD3	LMD4	LMA4	LMA5	LMA6	LMA7	LMA8	LMA9	LME3
J1-031	+	+++	+++	-	-	-	+	+++	+++	-
C1-056	+++	+++	-	+++	+++	+++	++	+++	+++	Host
J2-031	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
Scott A	+++	+++	+++	Host	Host	Host	Host	Host	Host	+
J1-094	+++	+++	-	+++	+++	+++	+++	+++	+++	++
J2-064	+++	Host	Host	+++	+++	+++	++	+++	++	+
J1-168	+	+++	+++	-	-	-	+++	++	++	-
C1-115	+++	-	-	+++	++	++	-	+++	++	+
R-2500	+++	-	-	+++	+++	+++	-	+++	++	++
51775	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
N3-031	Host	-	-	-	-	-	-	-	-	-

+++, EOP 1 to 0.5; ++, EOP 0.5 to 0.2; +, EOP 0.2 to 0.001; -, bacterial strain was not susceptible to phage attack

Summary I

- Epidemiology data does not seem to indicate a greater risk of organic foods
- Survey studies did not detect increased contamination
- Use of manure poses a greater risk than not using it
- Survival in manure is difficult to predict

Summary II

- Current organic manure practices should be reviewed
- There is great need to develop effective non-synthetic sanitizers/antimicrobial substances for organic food production
- Phages have great potential for control of specific pathogens

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Questions?