
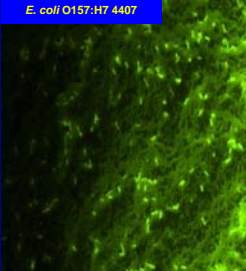


**Survival and Stress Response of
Escherichia coli Exposed to
Alkaline Cleaners**

Manan Sharma, Ph.D.
Environmental Microbial and Food
Safety Laboratory
USDA-Agricultural Research Service
Northwest Food Safety and Sanitation
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***Escherichia coli* O157:H7**




E. coli O157:H7 4407

- Causes 96,000 cases of infection each year
- 3200 hospitalization, 31 deaths annual in the U.S.
- Natural reservoir is cattle
- Variety of food commodities contaminated (meat, produce, juices)
- Survives under acidic conditions






Uses of alkaline cleaners




- Strongly alkaline cleaners (pH 12.7) are used in commercial ovens and smokehouses
- Heavy-duty cleaners (pH 10 - 12.0) are used in CIP and high pressure systems
- Mild alkaline cleaners (pH 9.7-10.1) are used to hand clean lightly soiled areas




Behavior of foodborne pathogens exposed to alkali

- *E. coli* O157:H7 may have higher resistance than non-pathogenic *E. coli* to extreme alkaline pH
- *Salmonella* Enteritidis has increased thermotolerance
- *Listeria monocytogenes* has increased tolerance to heat and bacteriocins
- Are bacterial strains inherently resistant to certain stresses, or can they be 'stress-hardened'?




Role of *rpoS* in *E. coli* O157:H7

- Plays a role in acid resistance of *E. coli* O157:H7 and *Salmonella*
- Has been postulated to play a role in allowing bacteria to survive the alternating acidic and alkaline conditions faced in gastrointestinal systems
- Several deaminases regulated by *rpoS* have shown increased expression in *E. coli* grown at alkaline pH




Objectives

- To determine the survival characteristics of cells of *E. coli* O157:H7 in logarithmic and stationary growth phases upon exposure to a variety of alkaline cleaners
- To evaluate stationary phase cells surviving exposure to alkaline cleaners for changes in thermotolerance and resistance to sanitizers.




Objectives

- To determine the survival characteristics of *E. coli* O157:H7 cells exposed to an alkaline cleaner and inoculated into sliced roast beef and hard salami followed by storage at various temperatures.
- To examine the *rpoS* gene for its role in protecting cells of *E. coli* O157:H7 treated with alkaline cleaners and potential cross protection of treated cells against subsequent exposure to heat, sanitizers, and survival in sliced roast beef and salami.



Objectives

- To determine the effectiveness of an alkaline cleaner and lytic bacteriophage in killing cells of *E. coli* O157:H7 in a biofilm formed on stainless steel.



SENSITIVITY OF *ESCHERICHIA COLI* O157:H7 TO COMMERCIALY AVAILABLE ALKALINE CLEANERS AND SUBSEQUENT RESISTANCE TO HEAT AND SANITIZERS

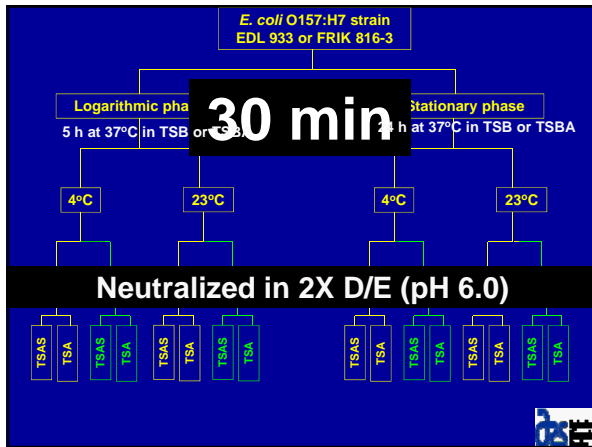


Formulation and pH of alkaline cleaners, NaOH, and KOH evaluated for lethality to *E. coli* O157:H7

Cleaner	Trade name	Active Ingredients	pH
1	ProChlor	KOH (< 10%), NaSiO ₂ (<10%), NaOCl (<10%)	11.31
2	FS 4089	KOH (< 10%), NaOH (<10%), NaOCl (<10%)	11.64
3	FS 4490	KOH (<10%), NaOCl (<10%)	11.35
4	K Foam SF Plus	KOH (<10%), NaOCl (< 5%)	11.20
5	K Foam Lo	ethylene glycol monobutyl ether	10.41

Formulation and pH of alkaline cleaners, NaOH, and KOH evaluated for lethality to *E. coli* O157:H7

Cleaner	Trade name	Active Ingredients	pH
6	Quorum Yellow	NaOH (10%), total available chlorine (2.5%)	11.31
7	Enforce	NaOH (11%), total available chlorine (1.8%)	11.69
NaOH		0.025 M	12.17
KOH		0.025 M	12.20



Populations (\log_{10} CFU/ml) of *E. coli* O157:H7 recovered on TSA as affected by treatment time.

Treatment	Strain	Treatment time (min)		
		2	10	30
Peptone (control) ¹	EDL 933	a 8.31 a	a 8.39 a	a 8.39 a
	FR1K 816-3	a 8.19 a	a 8.18 a	a 8.38 a
Cleaners ²	EDL 933	a 6.53 a	a 5.38 b	a 4.69 c
	FR1K 816-3	b 6.16 a	a 5.35 b	a 4.58 c

¹Mean values in the same row that are not followed by the same letter are significantly different ($P \leq 0.05$); within the same treatment and treatment time, mean values not preceded by the same letter are significantly different ($P \leq 0.05$).

²Values represent means over all growth phases and treatment temperatures.

³Values represent means over all growth phases, cleaners, cleaner concentrations and treatment temperatures.


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Populations (\log_{10} CFU/ml) of *E. coli* O157:H7 recovered on TSA as affected by cleaner or hydroxide solution, treatment, concentration, and temperature.

Treatment	Treatment temperature and concentration			
	4°C		23°C	
	25%	100%	25%	100%
Peptone (Control)	8.20 a	8.20 a	8.41 a	8.41 a
ProChlor	a 7.85 a	b 5.51 c	a 8.24 a	b 3.76 e
FS 4089	a 5.78 d	b 2.04 f	a 4.77 c	b 1.41 h
FS 4490	a 8.12 a	b 5.93 c	a 8.24 a	b 5.81 c
K Foam SF Plus	a .745 b	b 2.92 e	a 6.23 b	b 1.75 gh
K Foam Lo	a 8.13 a	b 7.58 b	a 8.06 a	b 6.96 b
Quorum Yellow	a 7.19 b	b 2.10 f	a 6.01 b	b 2.13 fg
Enforce	a 5.74 c	b 2.54 ef	a 4.45 c	b 2.55 f
NaOH (0.025 N)		4.63 d		4.51 d
KOH (0.025 N)		4.74 d		4.96 d



Populations (log₁₀ CFU/ml) of *E. coli* O157:H7 recovered on TSA as affected by cleaner or hydroxide solution, treatment, concentration, and temperature.

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KOH (0.025 N)		4.74 d		4.96 d

Conclusions on the exposure of *E. coli* O157:H7 to alkaline cleaners

- Cells of *E. coli* O157:H7 in logarithmic phase were not more sensitive to killing by alkaline cleaners than those in stationary phase.
- 12% and 15% of treated suspensions of EDL 933 and FRIK 816-3, respectively, showed higher counts on TSA than on TSAS.
- Reductions in populations of both strains at 4 and 23°C were similar.

Conclusions on the exposure of *E. coli* O157:H7 to alkaline cleaners

- Reductions in populations of EDL 933 and FRIK 816-3 were similar



Thermal treatment of *E. coli* O157:H7 after exposure to alkaline cleaners


E. coli O157:H7 strain EDL 933 or FRIK 816-3 in stationary phase

↓

Cell suspensions were exposed to control (0.05% Peptone), K Foam Lo, and Enforce at 4, 12 and 23°C for 2 min before neutralization, washing and resuspension in 0.05% peptone

↓

Suspensions were heated in capillary tubes submerged in a water bath at 55°C for up to 32 min.



Thermal treatment of *E. coli* O157:H7 after exposure to alkaline cleaners


Suspensions in capillary tubes were removed from the water bath at 0, 3, 6, 12, 15, 18, 24, 28, or 32 min.

↓

Contents of crushed capillary tubes were plated on TSA

↓


Thermal death times (min) were determined by plotting the best fit line of populations of *E. coli* O157:H7 against time and calculating D-values



D_{55°C} (min) of *E. coli* O157:H7 as affected by pre-treatment with cleaners.¹

Pre-treatment		D _{55°C} Value (min)		
Temp (°C)	Strain	Control	K Foam	Enforce
4	EDL 933	a 16.4 b	a 12.5 b	a 24.1 a
	FRIK 816-3	a 15.6 a	a 12.4 a	a 19.0 a
12	EDL 933	a 22.9 a	a 12.2 b	a 19.7 a
	FRIK 816-3	a 13.9 a	a 11.9 a	b 13.8 a
23	EDL 933	a 14.3 b	a 9.5 b	a 20.8 a
	FRIK 816-3	a 14.7 b	a 9.3 b	a 21.7 a


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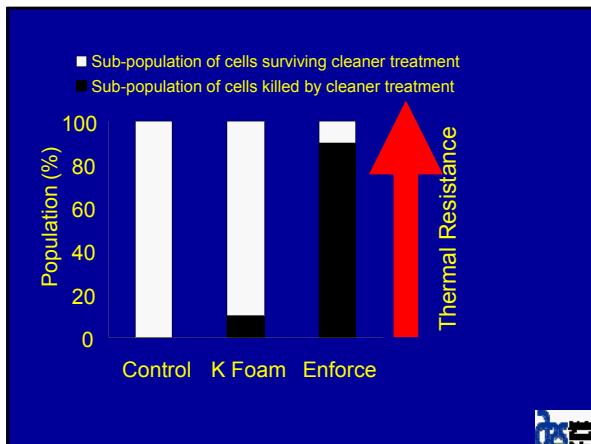


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	FRIK 816-3	a 13.9 a	a 11.9 a	b 13.8 a
23	EDL 933	a 14.3 b	a 9.5 b	a 20.8 a
	FRIK 816-3	a 14.7 b	a 9.3 b	a 21.7 a


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Treatment of *E. coli* O157:H7 with sanitizers after exposure alkaline cleaners

- Quorum Clear – quaternary ammonium compounds, used at 200 µg/ml
- Quorum Green – sodium hypochlorite, used at 200 µg/ml
- FS Amine B – quaternary ammonium compounds, used at 150 µg/ml
- Cetylpyridinium chloride – 100 µg/ml
- Benzalkonium chloride – 100 µg/ml



Treatment of *E. coli* O157:H7 with sanitizers after exposure alkaline cleaners


E. coli O157:H7 strain EDL 933 or FR1K 816-3 with in stationary phase

↓

Cell suspensions were pre-treated with control (0.05% Peptone), K Foam Lo, and Enforce at 4 and 12°C for 2 min before neutralization, washing and resuspension in 0.05% peptone

↓


Cell suspensions were exposed to control and sanitizer treatments at 4 and 12°C for 1 min before neutralization




Treatment of *E. coli* O157:H7 with sanitizers after exposure to alkaline cleaners

↓

Neutralized suspensions containing cells, sanitizer and 2X DE were plated on TSA and TSAS




SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN ROAST BEEF AND SALAMI AFTER EXPOSURE TO AN ALKALINE CLEANER



Survival of *E. coli* O157:H7 in roast beef and salami after exposure to alkaline cleaner


- Sliced roast beef (pH 6.3) and hard salami (pH 4.9)
- Two nalidixic acid resistant strains (EDL 933 and FRIK 816-3)
- Alkaline cleaner treatment (Enforce) and control (0.05% peptone) for 2 min.

Treatment	Inoculum of <i>E. coli</i> O157:H7	
	Low	High
Control	1:1000	undiluted
Enforce	1:10	undiluted

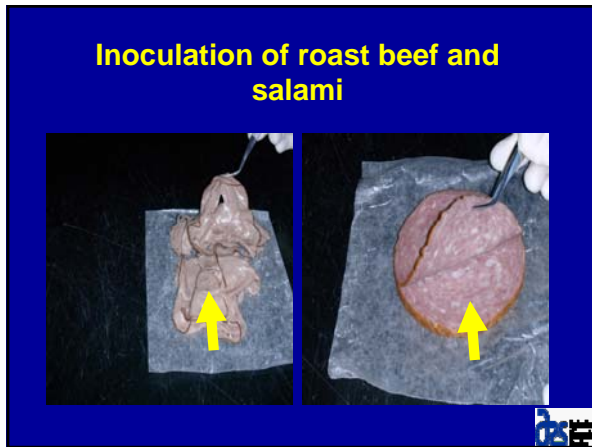


Survival of *E. coli* O157:H7 in roast beef and salami after exposure to alkaline cleaner

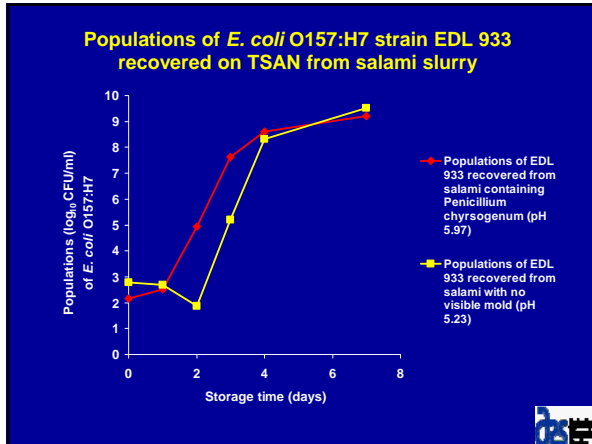
- 50 µl of inoculum applied to each sample of 50 g of roast beef or hard salami
- Storage temperatures: 4°C, 12°C, and 20°C (salami only) for up to 21 days in sterile filtered stomacher bags
- Enrichment with 100 ml of Universal Pre-enrichment broth
- Stomaching for 1 min.
- Recovery media used: SMACN and TSAN or SMACAN and TSAAN







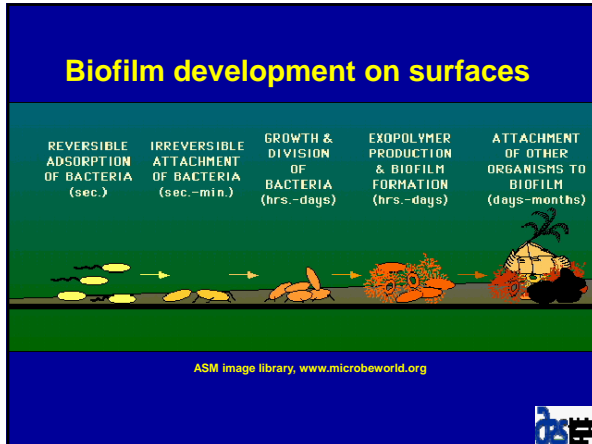


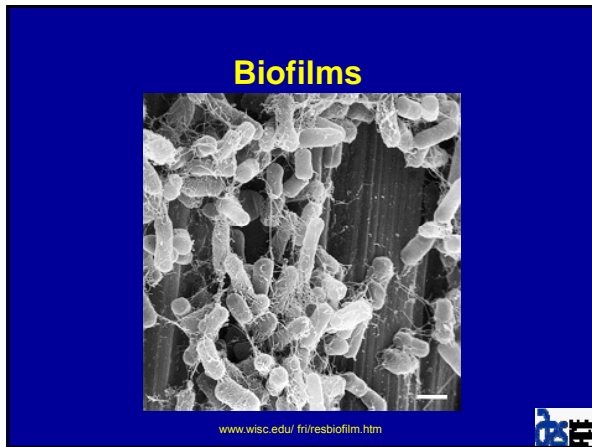


Conclusions on survival of *E. coli* O157:H7 pre-treated with Enforce and control in roast beef and salami

- Inocula of both strains treated with Enforce were resuscitated in roast beef stored at 12°C.
- Inocula of both strains treated with Enforce were not able to resuscitate in salami due to the low pH
- Inocula of cells treated with control surviving in roast beef at 4°C and in salami at 4, 12, and 20°C were temperature and pH stressed.

INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN BIOFILMS ON STAINLESS STEEL BY TREATMENT WITH ALKALINE CLEANER AND BACTERIOPHAGE





Preparation and treatment of biofilms of *E. coli* O157:H7 on stainless steel coupons

- Two strains – ATCC 43895 and FRIK 816-3
- Coupons were placed in an inoculum of ca. 8 log CFU/ml in PBS and stored at 4°C for 24 h
- Biofilms on coupons were then developed in MSB or MSBA and incubated at 22°C for 96 h with one transfer to fresh media

ASAP



Preparation and treatment of biofilms of *E. coli* O157:H7 on stainless steel coupons

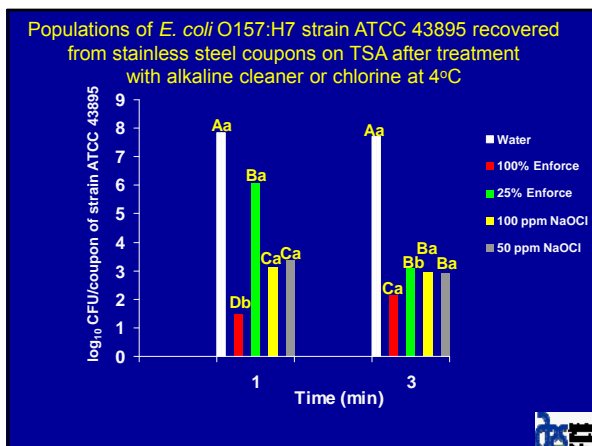
- Biofilms on coupons were treated at 4°C for 1 and 3 min with:
 - water (control)
 - 100% Enforce
 - 25% Enforce
 - 100 ppm free chlorine
 - 50 ppm free chlorine
- Coupons were then transferred to D/E broth

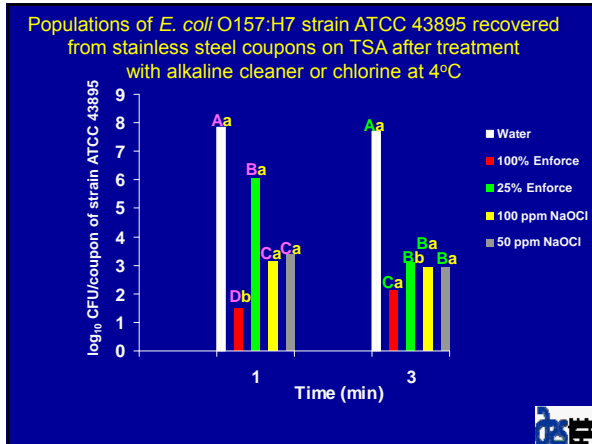


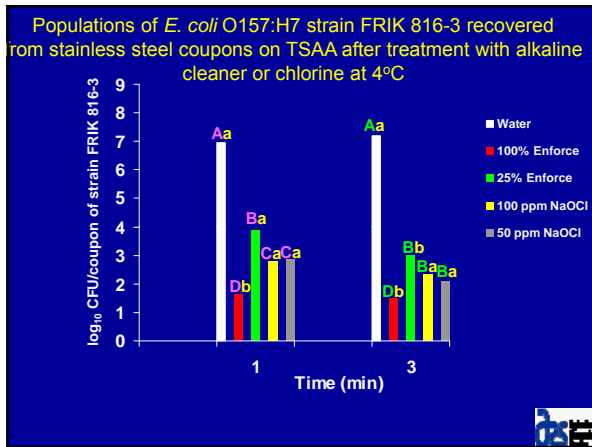

Preparation and treatment of biofilms of *E. coli* O157:H7 on stainless steel coupons

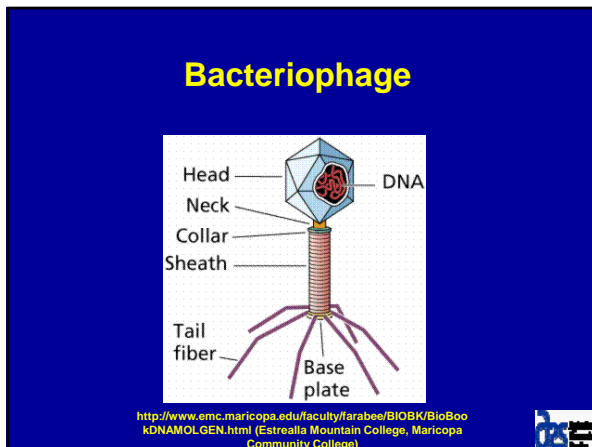
- Coupons in D/E were vortexed with glass for 1 min with glass beads
- Populations of ATCC 43895 and FRIK 816-3 were recovered by spiral plating on TSA and TSAA, respectively

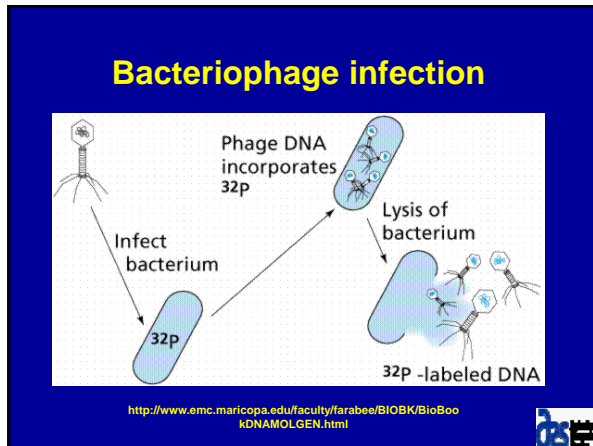







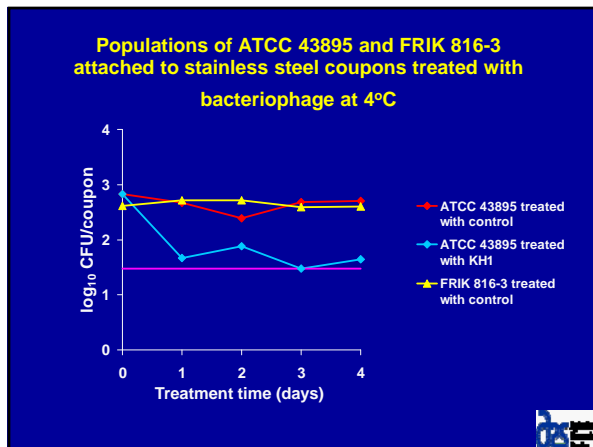


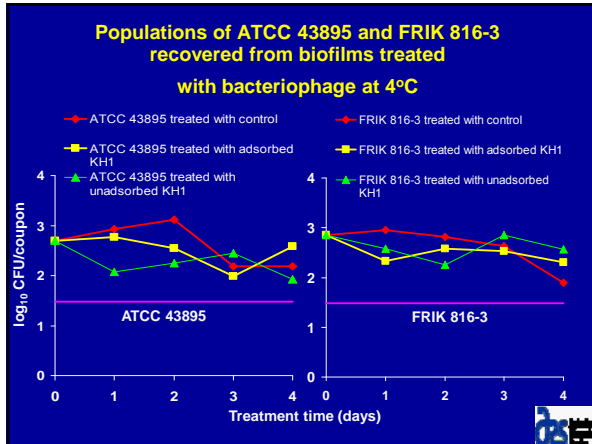


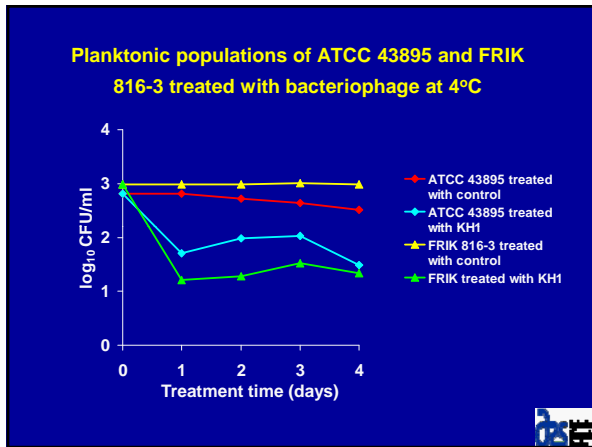


Treatment of cells of ATCC 43895 and FRIK 816-3 with bacteriophage

Types of cells treated with bacteriophage			
	Attached cells	Cells in biofilms	Planktonic cells
Preparation	Coupons placed in suspensions (4 log CFU/ml) at 4°C for 24 h	Cells attached and incubated in MSB with 5 mM MgSO ₄ or MSBA for 22°C for 24 h	Suspensions of cells (3 log CFU/ml) stored at 4°C for 24 h
Population	ca. 3 log CFU/coupon	ca. 3 log CFU/coupon	ca. 3 log CFU/ml
Bacteriophage treatment	8 log PFU/ml, adsorbed, in MB	7.5 log PFU/ml, adsorbed and unadsorbed, in MB	8 log PFU/ml, adsorbed, in PBS with 5 mM MgSO ₄
Storage conditions	4°C for up to 4 days		
Recovery and enumeration	Surface plating on TSA and TSAA		








Conclusions on treatment of biofilms with alkaline cleaner and bacteriophage

- Cells of *E. coli* O157:H7 in biofilms of both strains were killed more efficiently by treatment with an alkaline cleaner than by hypochlorite alone.
- Attached cells may be susceptible to killing by bacteriophage KH1, but cells in biofilms may not be as susceptible.


Conclusions

- Alkaline cleaners containing a combination of KOH or NaOH and hypochlorite were effective in reducing populations of *E. coli* O157:H7 in suspension and in biofilms, indicating a possible synergistic mechanism between alkaline pH and hypochlorite.
- Stationary phase cells of EDL 933 and FRIK 816-3 pre-treated with an alkaline cleaner containing sodium hydroxide and hypochlorite at 4 and 23°C had higher $D_{55^\circ\text{C}}$ values than those treated with cleaner not containing hydroxide or hypochlorite, indicating that they may be cross protected against heat.




Conclusions

- Pre-treatment of *E. coli* O157:H7 with alkaline cleaners, regardless of sodium hydroxide or hypochlorite content, did not cross protect cells against sanitizer treatments.
- Treatment of cells of *E. coli* O157:H7 with alkaline cleaner before inoculation into roast beef stored at 12°C did not prevent resuscitation.



Conclusions

- Treatment with alkaline cleaner did not cross protect cells of *E. coli* O157:H7 against cold stress imposed by roast beef at 4°C or acidic stress imposed by salami at all storage temperatures.
- The *rpoS* gene does not appear to protect cells from the bactericidal effects of alkaline cleaners or stresses imposed by inoculation in roast beef and salami.



Conclusions

- The *rpoS* may play a role in protecting cells from heat after alkaline cleaner treatment at 12°C or in allowing cells to resuscitate more rapidly in deli meat products stored at 12°C.
- Alkaline cleaners containing the same free chlorine content as hypochlorite solutions are more effective in reducing populations of *E. coli* O157:H7 in biofilms.
- Bacteriophage KH1 was not effective in killing cells in biofilms but was effective in reducing populations of *E. coli* O157:H7 attached to stainless steel surfaces.



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Thank



You