Biocontrol of Salmonella Associated with Sprouting Mung Beans using a Combination of Antagonistic Bacteria and Lytic Bacteriophages

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Sprouted Seeds

- Mung beans (bean sprouts)
- Alfalfa
- Soy bean
- 35 foodborne illness outbreaks
- Sprouts: Ideal growth medium for pathogens



Food Safety Interventions

Screening seeds for pathogens

Seed sanitation

Spent water testing

Spent Irrigation Water Testing

- Culture-free, integrated pathogen concentration and detection system for on-site testing.
- Concentration: Tangential Flow Filtration
- Detection: Flow-through Electrochemical Enzyme Linked Immuno-Assay.

Advances in Spent Irrigation Water Testing



TFF Cell Concentration







Verification Trials

Spent irrigation water derived from mung bean beds initially inoculated with 1.3 cfu/g Salmonella



- Large sample volumes (10 liters) taken from multiple sites.
- No delay in obtaining microbiological results
- Screening of samples later in the sprouting period.



Calcium Hypochlorite (20, 000ppm) Vs

Germin-8-or (200ppm)

Treatment of	E. coli (D157:H7	Salmonella		
mung beans	Count Log cfu/g	Enrichment	Count Log	Enrichment	
			cfu/g		
Calcium hypochlorite (20, 000ppm, 20mins)	8.59	NT	7.96	NT	
Germin-8-or 200ppm	ND	ND	ND	ND	

Initial loading: 3-4 log cfu/g

ND <1 cfu/25g

Germin-8-or

- Effective, low-cost, method for decontaminating seeds
- More effective than current methods

Currently seeking regulatory approval

Biocontrol

Restablished method for controlling plant pests *caBacillus thuringiensis* (BT) insecticide **Probiotics R**Bacteriophages [∞]Non-pathogenic Real No adverse effects on plant development *∝*Effective Real Adapted to the environment

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Approach

Antagonistic bacteria

Bacteriophages



Salmonella infecting Bacteriophages

Isolated from pig farms and feedlots

Cocktail of phages which infect broad range of serovars





Lytic Activity of Bacteriophage on Salmonella Serovar

Serovar/Phage Isolate	F01	P01	P102	P700	P800	FL 38	FL 41
Javiana	+	+	+	+	+	ND	ND
Heidelberg	+	+	+	+	+	ND	ND
Typhymurium	+	+	+	+	+	ND	ND
Newport	+	-	-	+	-	ND	ND
Montevideo	-	-	-	-	-	+	+

+ Susceptible

- Resistant

ND Not determined



Anti-Salmonella Factor

- No anti-Salmonella activity recovered in:-
 - Spent culture medium of *E. asburiae*
 - Elution from agar plates on which *E. asburiae* had been cultured.
 - Cell membrane fraction

Anti-Salmonella factor requires to be elucidated.

In Vitro (BROTH) Assay



Suppression of Salmonella growth in mung bean sprouts



Steep beans In *Salmonella* suspension Steep beans In *Enterobacter* Phage Sprouting for 5 days with daily irrigation





Control of Salmonella on sprouting mung bean sprouts

Treatment	Weight of Sprouts (g)	TVC ª (Log CFU/g)	Enterobacter asburiae JX1 (Log CFU/g)	Salmonella (Log CFU/g)
Negative Control	78.20 7.43A	8.15 0.91A	<1.70 A	Not Detected ^b A
Salmonella	69.98 7.96A	8.15 0.46A	<1.70 A	6.72 0.78B
Salmonella + E. asburiae	72.43 4.87A	8.48 0.56A	8.14 0.60B	1.16 2.14C
Salmonella + phage	70.60 8.27A	8.52 0.44A	<1.70 A	3.31 2.48C
<i>Salmonella</i> + <i>E. asburiae</i> + phage	71.33 9.06A	8.98 o.41A	7.91 0.16B	Positive ^c D

Microflora of Sprouted Seeds

1 2 3 4 5 6

- 1: Salmonella + Enterobacter + phages
- 2: Salmonella + bacteriophages
- 3: Salmonella + Enterobacter
- 4: Salmonella
- 5: Control (non-inoculated)
- 6: Salmonella culture

Alfalfa Sprouts

			Log cfu/g sprouts					
Treatment	Sprout Yield (seeds)	ts (g/25g	TAC		Entero	obacter	Salmo	onella
Control	91.0	12.0	8.15	0.09	ND		ND	
Salmonella	102.5	10.2	7.89	0.23	ND		7.62	0.21
Salmonella + Enterobacter + Phages	104.0	10.1	8.41	0.27	8.40	0.60	ND	

ND: Not Detected

Does the Same Approach Work with Other Pathogens?

Escherichia coli O157:H7

- Three bacteriophage cocktail from culture collection
- Enterobacter asburiae JX1
- In vitro assay

Growth of *E. coli* O157:H7 in Broth Culture



Listeria monocytogenes

- 3 strain cocktail of phages from collection
- Antagonistic bacteria
 - Manure effluent
 - Soil
 - Mung bean sprouts

Isolation of Antagonistic Bacteria

Code	Identification	Source
BA	Bacillus pumilus	Effluent
BB	Bacillus lentus	Effluent
BC	Bacillus pumilus	Effluent
BD	Bacillus subtilis	Effluent
BE	Bacillus cereus	Soil
BF	Bacillus megaterium	Soil
PA	Bacillus cereus	Soil
PB	Bacillus cereus	Effluent
EA	Enterobacter asburiae	Sprouts

In Vitro Assay



Conclusions

cRE. asburiae JX1 exhibited antagonistic activity against Salmonella

Combination of *E. asburiae* JX1 and lytic bacteriaphage cocktail exhibited synergistic activity against *Salmonella* associated with sprouting seeds.

Complementary or alternative to seed decontamination

Generic approach to control foodborne pathogens although requires further optimization.

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Selected Publications

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- McEgan, R*; Fu, TJ; Warriner, K (2009). Concentration and Detection of Salmonella in Mung Bean Sprout Spent Irrigation Water by Use of Tangential Flow Filtration Coupled with an Amperometric Flowthrough Enzyme-Linked Immunosorbent Assay. J. Food Prot 72; 591-600.
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