

Project Start date: August 1, 2006
 Project duration: 2 years

Project Title:
Multipathogen Screening Using Immunomicroarray

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Overall Objective

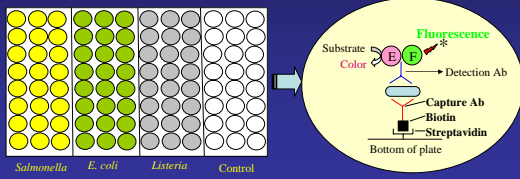
- Immunomicroarray for concurrent detection of viable *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella enterica*.

Specific Objectives

- Microarray assay in 96-well plate and glass slide using sandwich format
- Growth and enrichment of three pathogens (healthy or stressed) spiked in model food samples in a selective enrichment broth for use with microarray.

Multipathogen Screening

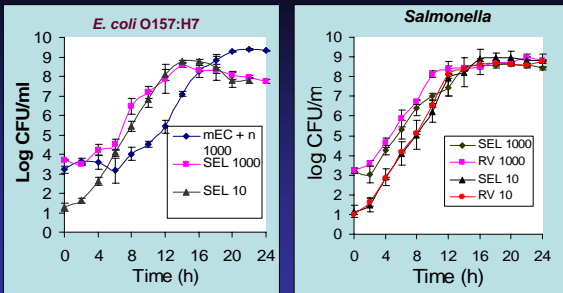
Test sample
 ↓
 Enrichment in PEDD using universal selective enrichment broth (SEL broth)
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 Microarray
 96-well plate/Glass slide



Objective 2: to optimize growth and enrichment of three pathogens (healthy or stressed) spiked in model food samples in a selective enrichment broth for use with microarray.

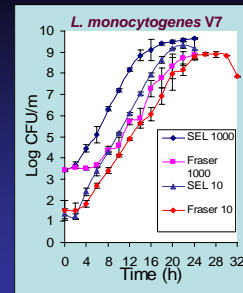
- SEL formulation for Salmonella, E. coli and Listeria (completed)
- Growth profiles of target pathogens
- Growth inhibition of natural microbes
- Growth of stressed bacteria
- Validation of SEL with immunoassay and PCR
- Comparison of performance with UPB (universal Pre-enrichment broth)
- Validation with spiked food samples

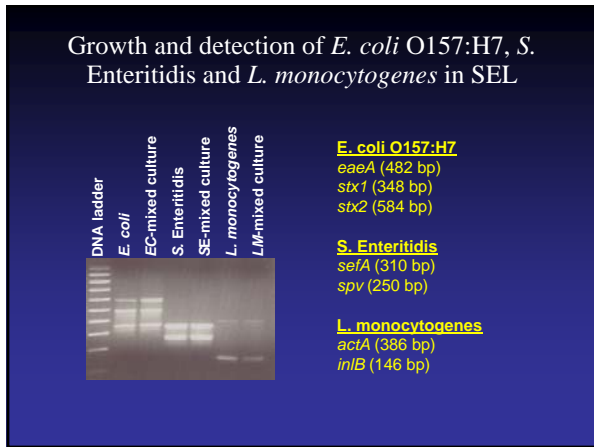
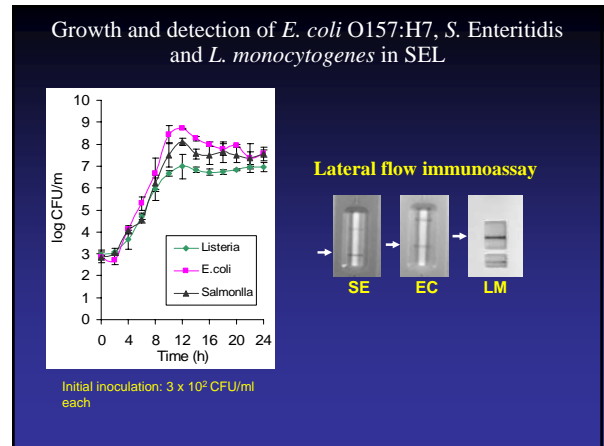
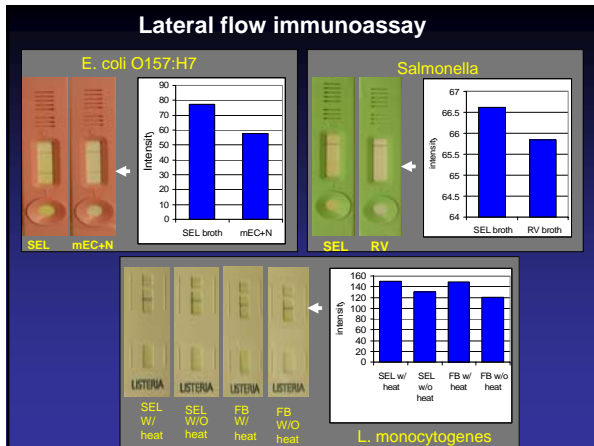
Growth in SEL



No growth of *E. coli* O157:H7 at 10 CFU/ml inoculation in m EC + n

Growth in SEL

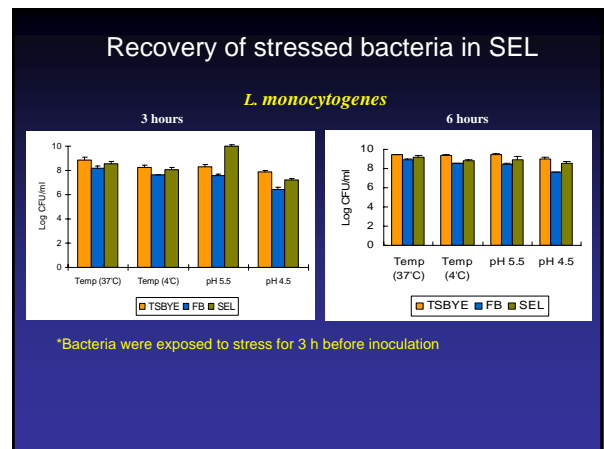
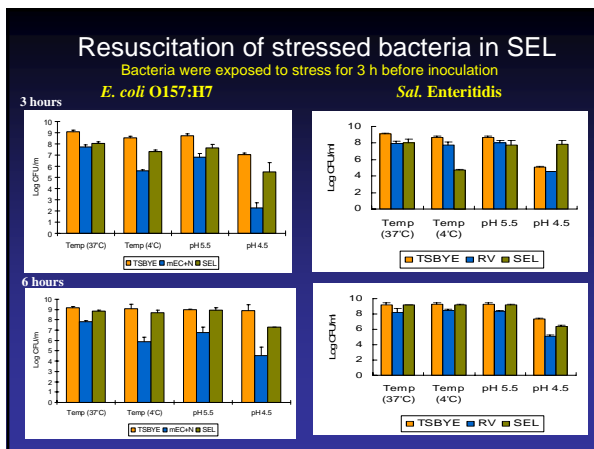




Growth of other cultures in SEL media

Culture	Growth by 24 h (OD ₅₉₅)
<i>Bacillus cereus</i> MS1-9	0.866 B*
<i>Bacillus megatarium</i>	0.006 D
<i>Bacillus subtilis</i>	0.008 D
<i>Enterobacter aerogenes</i>	1.390 A
<i>Enterococcus faecalis</i>	0.280 D
<i>Escherichia coli</i> O157:H7	0.890 B
<i>Lactobacillus rhamnosus</i>	0.014 D
<i>Listeria monocytogenes</i> V7	1.167 A
<i>Salmonella</i> Enteritidis	0.840 B
<i>Streptococcus mutans</i>	1.310 A
<i>Pseudomonas aeruginosa</i>	0.697 C
<i>Proteus vulgaris</i>	0.013 D

* Letters mean statistical differences (P<0.05)



Lateral flow immunoassay & PCR confirmation of pathogens in spiked meat grown in UPB and SEL

Turkey

Time (h)	<i>Listeria</i>		<i>Salmonella</i>		<i>E. coli</i>	
	UPB	SEL	UPB	SEL	UPB	SEL
8	-	±	-	-	++	++
10	+	+	-	-	++	++
12	±	+	±	±	++	++
16	+	+	+	+	++	++
24	++	++	±	±	++	++

Salami

Time (h)	<i>Listeria</i>		<i>Salmonella</i>		<i>E. coli</i>	
	UPB	SEL	UPB	SEL	UPB	SEL
8	-	-	-	-	++	++
10	-	-	-	-	++	++
12	-	±	-	-	++	++
16	++	+	-	-	++	++
24	++	++	±	±	++	++

Summary of SEL

- SEL supports growth of *Listeria*, *E. coli* and *Salmonella* together
- Inhibits select microbes
- Resuscitate stressed bacteria
- Suitable for antibody-and nucleic acid based (PCR) detection of pathogens from food
- Performance is superior to respective enrichment broths and comparable to the universal preenrichment broth (UPB)