PURDUE UNIVERSITY

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

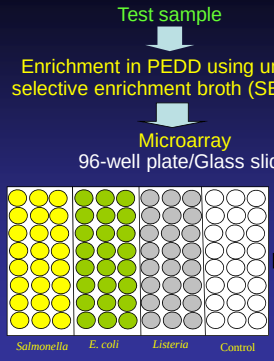
- Microarray assay in 96-well plate and glass slide using *sandwich format.*
- Growth and enrichment of three pathogens (healthy or stressed) spiked in model food samples in a *selective enrichment broth* for use with microarray.

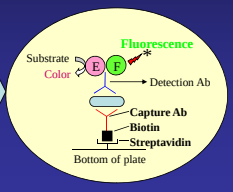
Multi-pathogen Screening

Test sample

Enrichment in PEDD using universal selective enrichment broth (SEL broth)

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

Objectives addressed during this reporting period

- Growth patterns of three pathogens inoculated at variable proportions in SEL
- SEL as enrichment broth for meat samples
- Detection using lateral flow immunoassay and PCR
- Effect of SEL on protein expression profiles (1D and 2D analysis)
- Identification of proteins
- Reaction characteristics of antibodies to pathogens grown in different enrichment broths

Validation of SEL performance using spiked food samples

Salami & deli turkey

Spiked with Ec/Lm/Sal
(~3 x 10^2 CFU/g each)

SEL (12h and 24h, 37 °C)

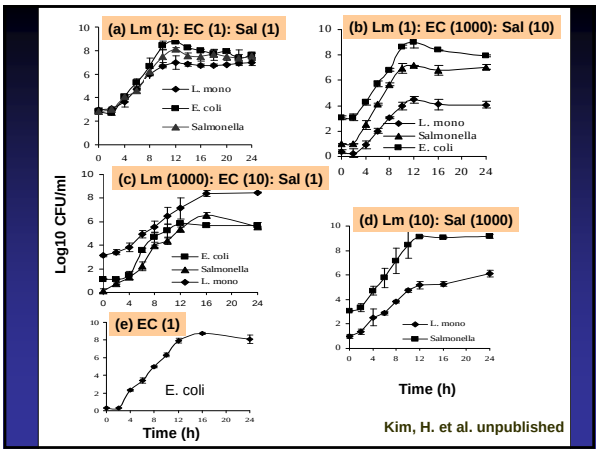
Plate Count
Ec: CT-SMAC
Lm: MOX
SE: XLD

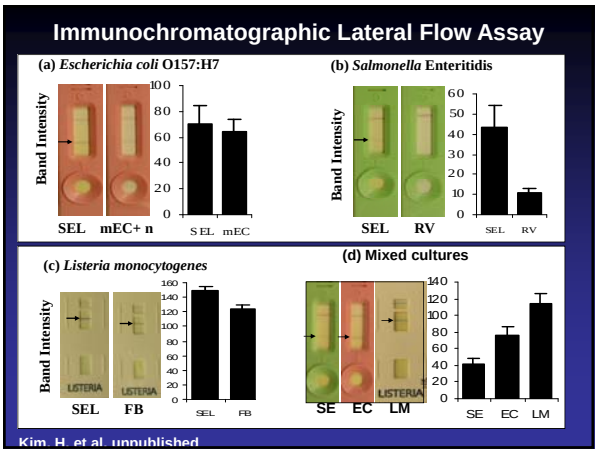
Lateral flow immunoassay

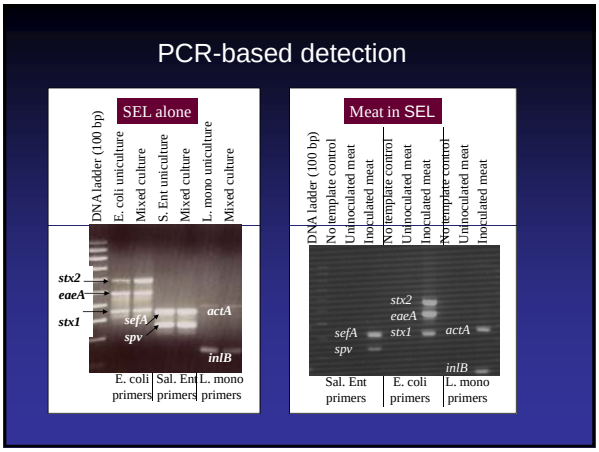
PCR

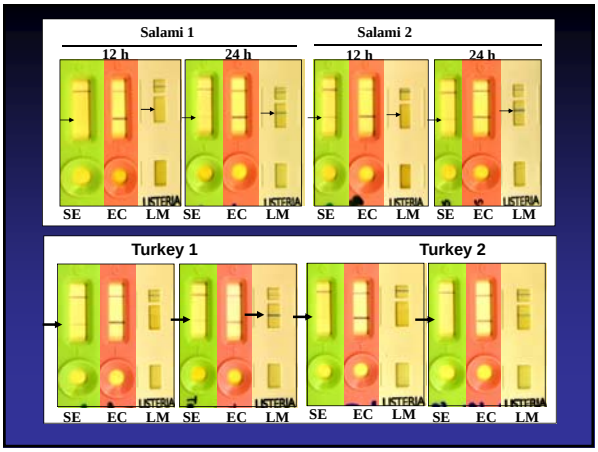
Viable counts (24 h)

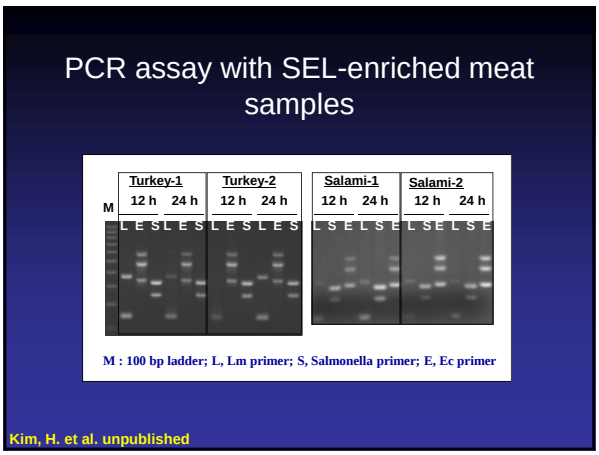
Food / Bacteria (Media)	Turkey	Salami
LM (MOX)	7.38 ± 0.05	6.66 ± 0.02
SE (XLD)	8.46 ± 0.13	7.53 ± 0.09
EC (CT-SMAC)	9.19 ± 0.06	8.22 ± 0.04

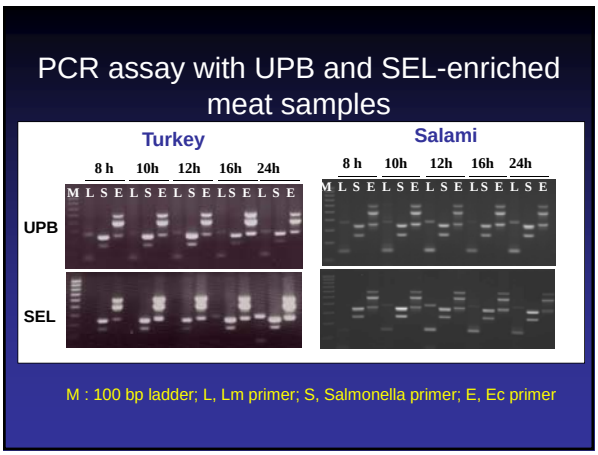

(a) Lm (1): EC (1): Sal (1)
(b) Lm (1): EC (1000): Sal (10)
(c) Lm (1000): EC (10): Sal (1)
(d) Lm (10): Sal (1000)
(e) EC (1)
L. mono
E. coli
Salmonella
Log10 CFU/ml
Time (h)
Kim, H. et al. unpublished


Immunochromatographic Lateral Flow Assay
(a) Escherichia coli O157:H7
(b) Salmonella Enteritidis
(c) Listeria monocytogenes
(d) Mixed cultures
Band Intensity
SEL
mEC+ n
RV
FB
SE
EC
LM
Kim, H. et al. unpublished


PCR-based detection
SEL alone
Meat in SEL
DNA ladder (100 bp)
E. coli uniculture
Mixed culture
S. Ent uniculture
L. mono uniculture
No template control
Uninoculated meat
Inoculated meat
stx2
eaeA
stx1
sefA
spv
actA
inlB
E. coli primers
Sal. Ent primers
L. mono primers

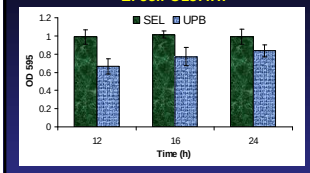

Salami 1
Salami 2
12 h
24 h
Turkey 1
Turkey 2
SE
EC
LM
LISTERIA


PCR assay with SEL-enriched meat samples
Turkey-1
Turkey-2
Salami-1
Salami-2
12 h
24 h
M
M : 100 bp ladder; L, Lm primer; S, Salmonella primer; E, Ec primer
Kim, H. et al. unpublished

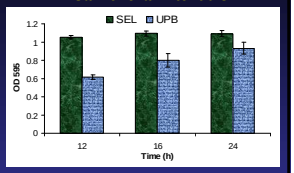

PCR assay with UPB and SEL-enriched meat samples
Turkey
Salami
8 h
10h
12h
16h
24h
UPB
SEL
M : 100 bp ladder; L, Lm primer; S, Salmonella primer; E, Ec primer

Comparison of bacterial growth between SEL and UPB

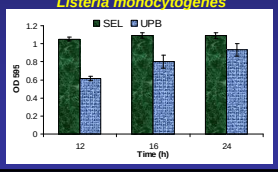
E. coli O157:H7



Salmonella Enteritidis



Listeria monocytogenes



Inoculation level: ca. 10^3 CFU/ml

Cultures	Source/Ref	ribotype	Growth in SEL			Growth in UPB		
			12 h	16 h	24 h	12 h	16 h	24 h
Escherichia coli								
O157:H7 EDL933 (EHEC)	FDA*	DUP-3064	1.01 ±0.08	1.00 ± 0.04	0.96 ± 0.08	0.67 ± 0.08	0.77 ± 0.10	0.84 ± 0.07
O157:H7 G5244 (EHEC)	CDC		1.00 ± 0.08	0.99 ± 0.07	0.97 ± 0.12	0.65 ± 0.06	0.78 ± 0.11	0.87 ± 0.14
O157:H7 G5286 (EHEC)	CDC		0.99 ± 0.09	1.03 ± 0.09	1.00 ± 0.10	0.67 ± 0.09	0.80 ± 0.12	0.92 ± 0.14
O157:H7 G5293 (EHEC)	CDC		0.94 ± 0.00	0.96 ± 0.00	0.95 ± 0.00	0.60 ± 0.02	0.68 ± 0.00	0.85 ± 0.07
O157:H7 G5303 (EHEC)	CDC		0.98 ± 0.04	1.01 ± 0.04	0.96 ± 0.03	0.61 ± 0.00	0.80 ± 0.02	0.86 ± 0.06
O157:H7 G5324 (EHEC)	CDC		0.98 ± 0.09	1.08 ± 0.04	0.97 ± 0.09	0.65 ± 0.05	0.77 ± 0.05	0.95 ± 0.02
O157:H7 C7927 (EHEC)	Apple cider isolate		1.03 ± 0.04	1.04 ± 0.06	1.03 ± 0.06	0.63 ± 0.01	0.76 ± 0.06	0.89 ± 0.02
O25:K98:NM (ETEC)	M. Donneberg	DUP-18656	0.04 ± 0.02	0.37 ± 0.04	0.52 ± 0.06	0.62 ± 0.06	0.77 ± 0.08	0.88 ± 0.13
O78:H11 (ETEC)	M. Donneberg	DUP-19199	0.00 ± 0.00	0.07 ± 0.09	0.00 ± 0.00	0.63 ± 0.06	0.82 ± 0.00	0.94 ± 0.12
O127:H6 ATCC 35401 (EPEC)		DUP-3017	0.96 ± 0.09	1.03 ± 0.06	1.00 ± 0.10	0.67 ± 0.06	0.75 ± 0.09	0.87 ± 0.06
O142:H6 ATCC 43886 (EPEC)			0.09 ± 0.02	0.29 ± 0.13	0.40 ± 0.06	0.63 ± 0.00	0.71 ± 0.00	0.87 ± 0.00
K12 (nonpathogenic)	Our collection		1.05 ± 0.07	1.13 ± 0.05	1.11 ± 0.09	0.63 ± 0.10	0.80 ± 0.08	0.86 ± 0.09
Listeria monocytogenes								
V7 (1/2a)	USFDA, dairy	DUP-1039	0.09 ±0.02	0.69 ± 0.05	0.91 ± 0.01	0.13 ± 0.01	0.47 ± 0.01	0.42 ± 0.01
Scott A (4b)	USFDA, Human,	DUP-1042	0.07 ± 0.00	0.73 ± 0.01	1.03 ± 0.00	0.40 ± 0.02	0.46 ± 0.01	0.44 ± 0.01
F4244 (4b)	CDC, human	DUP-1044	0.03 ± 0.00	0.59 ± 0.01	0.94 ± 0.05	0.36 ± 0.08	0.36 ± 0.05	0.31 ± 0.05
F4260 (1/2b)	CDC, human	DUP-1042	0.13 ± 0.00	0.94 ± 0.01	1.09 ± 0.02	0.43 ± 0.00	0.42 ± 0.01	0.41 ± 0.00
Listeria innocua		DUP-1006	0.19 ±0.02	0.97 ± 0.02	1.19 ± 0.02	0.43 ± 0.00	0.43 ± 0.00	0.42 ± 0.00
Salmonella enterica								
Enteritidis PT1		DUP-2035	1.05 ± 0.02	1.09 ± 0.03	1.09 ± 0.03	0.62 ±0.02	0.80 ± 0.07	0.93 ± 0.07
Kentucky 1271-94			0.95 ± 0.04	1.05 ± 0.02	1.07 ± 0.01	0.50 ± 0.04	0.70 ± 0.07	0.85 ± 0.11
Tennessee 825-94			0.99 ± 0.01	1.12 ± 0.01	1.19 ± 0.03	0.47 ± 0.05	0.73 ± 0.05	0.88 ± 0.05
Typhimurium	Our collection	DUP-1167	1.01 ± 0.03	1.10 ± 0.01	1.15 ± 0.01	0.72 ± 0.02	0.84 ± 0.04	1.00 ± 0.06

Comparison of competitive growth between SEL and UPB

Cultures	Growth in SEL (OD595)			Growth in UPB (OD595)		
	12 h	16 h	24 h	12 h	16 h	24 h
Bacillus cereus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.01	0.43 ± 0.00	0.48 ± 0.00
B. megaterium	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.00	0.47 ± 0.00	0.57 ± 0.01
B. subtilis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.21 ± 0.02	0.18 ± 0.00	0.18 ± 0.00
Enterococcus faecalis	0.00 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.45 ± 0.01	0.54 ± 0.01	0.48 ± 0.00
B. thermophilus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pseudomonas aeruginosa	0.15 ± 0.04	0.29 ± 0.02	0.37 ± 0.06	0.09 ± 0.05	0.28 ± .01	0.56 ± 0.01
Hafnia alvei	0.57 ± 0.11	0.93 ± 0.06	0.80 ± 0.07	0.62 ± 0.06	0.74 ± 0.06	0.86 ± 0.08
Serratia marcescens	0.03 ± 0.02	0.21± 0.06	0.83 ± 0.06	0.51 ± 0.01	0.70 ± 0.07	0.71 ± 0.05
Streptococcus mutans	0.32 ± 0.01	0.89 ± 0.10	0.84 ± 0.07	0.51 ± 0.01	0.66 ± 0.01	0.80 ± 0.01
Enterobacter aerogenes	1.11 ± 0.04	1.17 ± 0.03	1.23 ± 0.01	0.82 ± 0.03	0.93 ± 0.01	1.14 ± 0.10

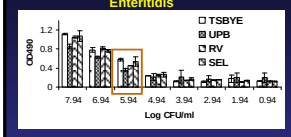
Kim, H. et al. unpublished

Comparison of growth of natural food isolates between SEL and UPB

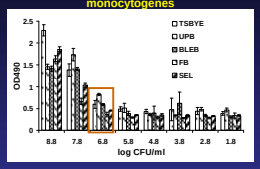
Natural food isolates	Growth in SEL (OD595)			Growth in UPB (OD595)		
	12 h	16 h	24 h	12 h	16 h	24 h
Bacillus megaterium HK1	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.00	0.50 ± 0.04	0.59 ± 0.06
Staphylococcus epidermidis HK7	0.21 ± 0.04	0.74 ± 0.04	0.65± 0.00	0.48 ± 0.09	0.69 ± 0.00	0.82 ± 0.02
Enterobacter cloacae HK8	1.06 ± 0.04	1.15 ± 0.05	1.13 ± 0.07	0.75 ± 0.01	0.85 ± 0.00	0.98 ± 0.00
Latococcus lactis ssp. *lactis* 21	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.00	0.33 ± 0.00	0.32 ± 0.00
Pediococcus acidilactici MRS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.01	0.10 ± 0.00	0.21 ± 0.00

Reaction characteristics of antibodies against pathogens grown in various enrichment broths

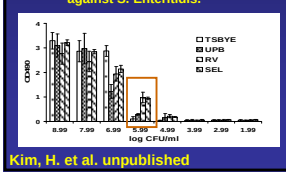
MAb-2F11 (1:600) against *Salmonella* Enteritidis



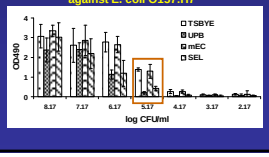
MAb C11E9-HRP (1:1200) against L. monocytogenes



KPL anti-Salmonella CAS-1 (1:5000) against S. Enteritidis.

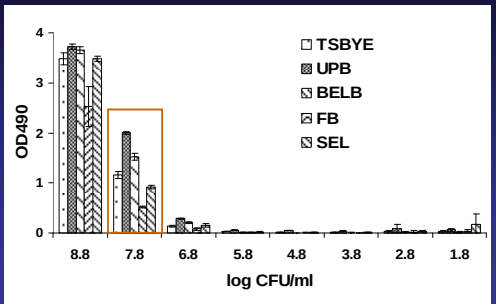


KPL Anti–E. coli O157:H7 (1:5000) against E. coli O157:H7

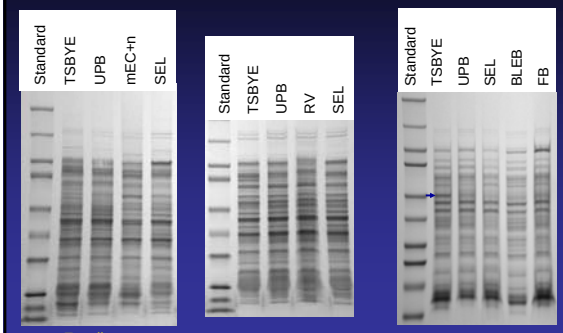


Kim, H. et al. unpublished

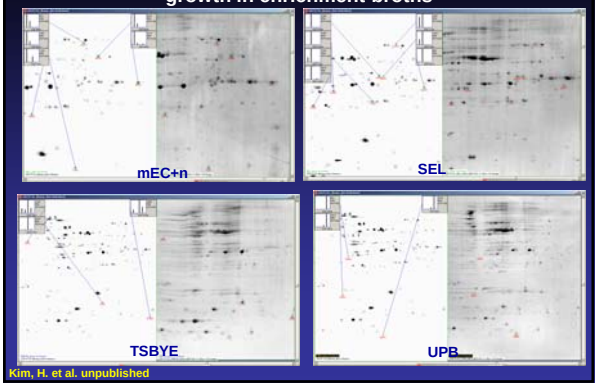
PAb-P66 (1:5000) against *L. monocytogenes*



SDS-PAGE analysis of protein expression profiles in pathogens



2D analysis of protein expression profiles in E. coli after growth in enrichment broths



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Unique proteins in *E. coli* O157:H7 from each medium

ID	media	protein	MW
7107	TSBYE	chain A, the crystal structure of Dps, A ferritin homolog that binds and protects Dna	18757
4410	TSBYEa	elongation factor Ts	30518
2206	TSBYEa	COG2310: uncharacterized proteins involved in stress response homolog of TerZ and putative cAMP-b	18772
4107	TSBYEa	COG3445: Acid-induced glycyl radical enzyme	14317
8404	TSBYEa	cysteine synthase A	34922
7406	TSBYEa	UDP-galactose-4-epimerase	37386
9402	TSBYEa	Glyceraldehyde-3-phosphate dehydrogenase	36104
3404	UPB	Hypothetical protein ECs2431	32682
3203	UPB	Thio peroxidase	17995
6202	mEC+n	chain A, purine nucleoside phosphorylase	26030
7101	mEC+n	chain A, the crystal structure of Dps, A ferritin homolog that binds and protects Dna	18757
8504	mEC+n	serine hydroxymethyltransferase	45487
9403	mEC+n	Glyceraldehyde-3-phosphate dehydrogenase	36104
6509	SEL	COG0031: cysteine synthase	34541
4409	SEL	Elongation factor Ts	30518
3302	SEL	COG0582: intergrase	39046
7705	SEL	pyruvate kinase I	59121
7403	SEL	COG0031: cysteine synthase	34541
8415	SEL	COG4150:ABC type sulfate transport system: periplasmic components	37647

Summary

- SEL supports growth of *Listeria, E. coli* and *Salmonella* together inoculated at variable proportions and is suitable for enrichment of pathogens in food for detection by antibody-or nucleic acid based (PCR) methods
- Inhibits select microbes
- Performance is superior to respective enrichment broths and comparable to the universal preenrichment broth (UPB)
- Antibody reaction profile is variable depending on the media
- 2D and MS analysis revealed variation in protein expression profiles