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Multipathogen screening and /or confirmation via microarray detection

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CFSE Annual Review – Oct 12-13, 2006

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Primary objective – to develop microarray based platforms to detect three major pathogens (*Listeria*, *Salmonella*, *E. coli*) simultaneously

Focus

1. Nucleic acid –based platform – Applegate (PI)
2. Antibody- based platform – Bhunia (PI)

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Immunomicroarray for multipathogens

Essentials

- Specific antibodies and immune reaction (Objective 1 & 1b)
- Platforms: Fiber optic and 96-well/slide (Objective 2)
- Sample preparation strategy-PEDD (Objective 3)
- Selective growth media (Objective 4)

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Objective 1: Antibody and antibody reaction

- Antibody Development:** Genomic and proteomic approaches in antibody development for *Listeria*, *E. coli* and *Salmonella*.
- Antigen Expression:** Effect of stress responses (media and environments) on antigen expression and immunoassays

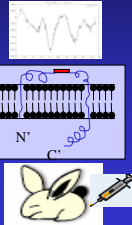
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Objective 1a: Genomic and proteomic approaches in antibody development for *Listeria*, *E. coli* and *Salmonella*

Strategies

- Search for species-specific surface exposed amino acid sequence
- Determine hydropathy profiles, surface localization, and antigenic properties
- Synthesize candidate peptide (15-20 amino acid) antigen
- Immunize rabbits and test for antibody production

5'-TCC-CGT-ACT-GAC-ATT-CTC-3'
N'- S - R - T - D - I - L -C'



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Antibody List

Antibody	Target antigen	Pathogen specificity	Antibody	Target antigen	Pathogen specificity
<i>E. coli</i>-specific antibodies			<i>Listeria</i>-specific antibodies		
Anti- <i>E. coli</i> O157:H7 PAb	LPS	<i>E. coli</i> O157:H7	C11E9 MAb	66 kDa	<i>L. mono</i> , <i>L. innocua</i>
Anti- <i>E. coli</i> PAb	Whole cells	<i>E. coli</i>	EM-7G1 MAb	66 kDa	<i>L. mono</i>
Anti- <i>E. coli</i> O157:H7 PAb	Intimin	<i>E. coli</i> O157:H7	EM-6E11 MAb	97 and 43 kDa	<i>Listeria</i> spp
<i>Salmonella</i>-specific antibodies			P66 PAb	66 kDa	<i>Listeria</i> spp
2F11 MAb	LPS	<i>Sal. Enteritidis</i>	MAb-EM10	104 kDa	<i>Listeria</i> spp
ST3229 PAb	32 kDa	<i>Sal. Typhimurium</i>	Anti- <i>Listeria</i> PAb	68, 62, 58, 50, 43 and 30 kDa	<i>Listeria</i> spp
ST3230 PAb	32 kDa	<i>Sal. Typhimurium</i>	Lm404 PAb	63 kDa IntB	<i>L. mono</i>
ST3236 PAb	205 kDa	<i>Sal. Typhimurium</i>	Lm407 PAb	52 kDa	<i>Listeria</i> spp
ST3238 PAb	25 kDa	<i>Sal. Typhimurium</i>	LmC639 PAb	90 kDa ActA	<i>L. mono</i>
Anti- <i>Salmonella</i> PAb	Multiple proteins	<i>Sal. Enteritidis</i>			
Anti- <i>Salmonella</i> CSA-1 PAb	Multiple proteins	<i>Sal. Typhimurium</i>			

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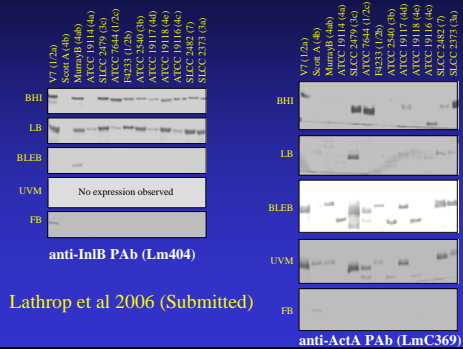
Objective 1b: Effect of stress responses on antigen expression and immunoassays

Motivation: As biosensors are becoming increasingly sensitive, a slight reduction in immune reactions due to "cellular or environmental factors" will result in "false" results

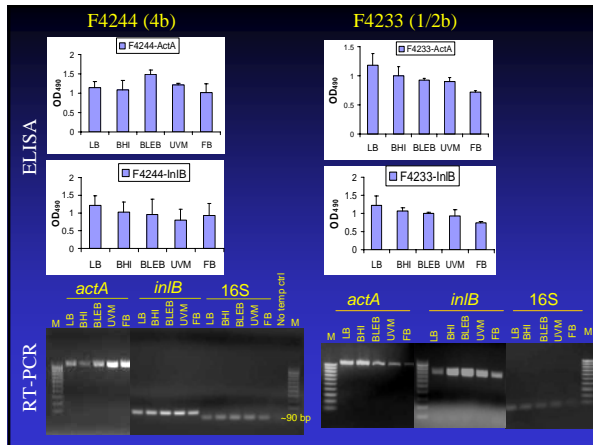
- Effect of selective enrichment broths on InlB, ActA and several unknown antigens in *L. monocytogenes*
- Effect of environmental stresses on antibody based detection of *E. coli* O157:H7, *S. enterica* and *L. monocytogenes*

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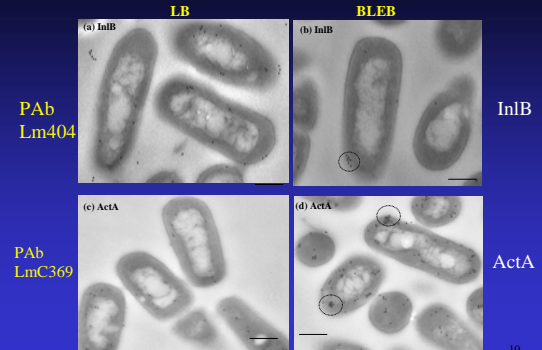
Effect of selective enrichment media on InlB and ActA expression for *L. mono*-specific antibodies



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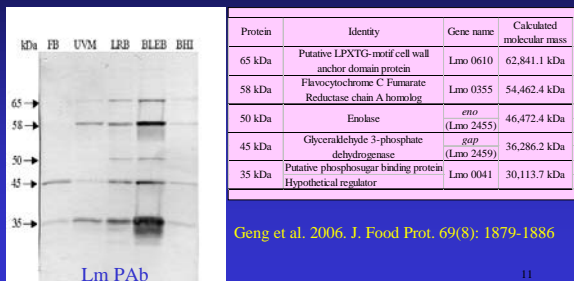


Transmission electron microscopic (TEM) analysis of InlB and ActA expression in different media



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Effect of enrichment broth on antigen expression for a *Listeria* specific PAB



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Effect of environmental stresses on antibody based detection of *E. coli* O157:H7, *S. enterica* and *L. monocytogenes*

Stress conditions investigated

- Heat 45°C; Cold (4°C); Acid (pH 5.0); Salt (NaCl- 5.5%), oxidative stress (H₂O₂; 15 mM, ethanol (5%)

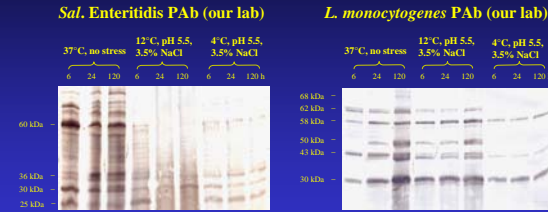
Duration

- Short-term (3h)
- Long term (>6 h)

Hahm and Bhunia. 2006. J. Appl. Microbiol. 100:1017-1027

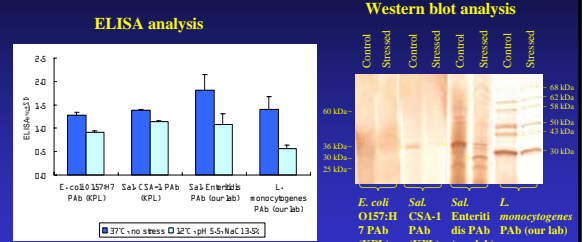
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Western blot analysis of antigen expression with long-term combined stress



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Examination of antibody reactions with bacteria grown in hotdogs in the presence of stress



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Summary: ELISA response to environmental stress

Antibody	Stress		ELISA response (Compared to control -37°C)
	Stress	Stress	
<i>E. coli</i> O157:H7 PAB (KPL)	Short-term (3 h)	4°C	16% ↓
		45°C	12% ↓
		5.5% NaCl	15% ↓
	Long-term and combined (pH 5.5, 3.5% NaCl)	4°C up to 6 h	13% ↓
<i>Sal.</i> CSA-1 PAB (KPL)	Long-term and combined (pH 5.5, 3.5% NaCl)	12°C up to 6 h	33% ↓
		4°C	34% ↓
	Short-term (3 h)	4°C	34% ↓
<i>Sal.</i> Enteritidis PAB	Short-term (3 h)	4°C	48% ↓
		45°C	37% ↓
		5.5% NaCl	30% ↓
		15 mM H ₂ O ₂	20% ↓
	Long-term and combined (pH 5.5, 3.5% NaCl)	4°C up to 120 h	32% ↓
<i>L. monocytogenes</i> PAB	Short-term (3 h)	pH 5.5	44% ↑
		5.5% NaCl	100% ↑
		5% ethanol	53% ↑
		45°C	53% ↑
	Long-term and combined (pH 5.5, 3.5% NaCl)	4°C up to 120 h	40% ↓
		4°C or 12°C up to 24 h	8-30% ↑

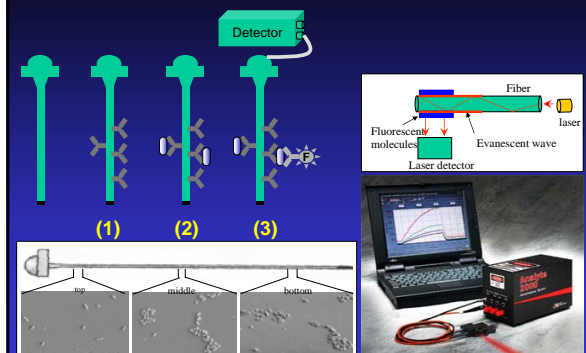
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Objective 2: Fiber optic sensor for food pathogens

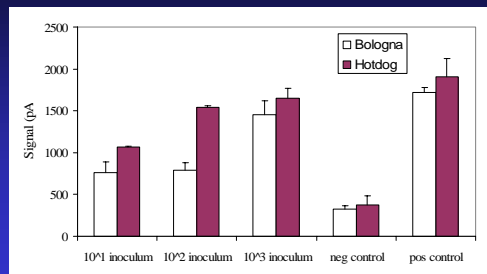
- *Listeria monocytogenes*
- *E. coli* O157:H7
- *Salmonella* Enteritidis

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Fiber optic sensor for pathogen detection



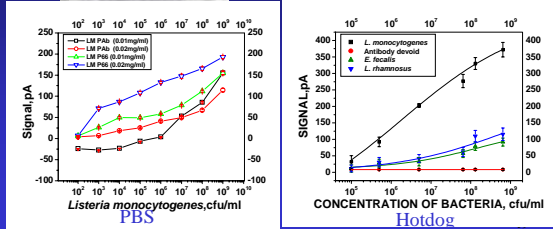
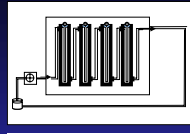
FO based detection of *L. monocytogenes* from inoculated meat samples – after 20 h-enrichment



Geng et al. 2004 Appl. Environ. Microbiol. 70:6138-6146

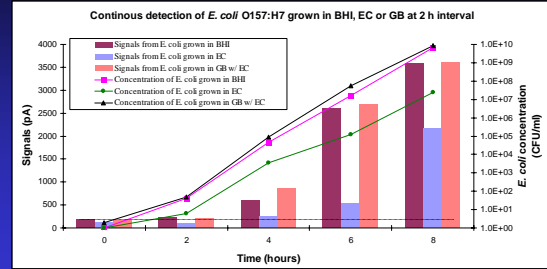
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L. monocytogenes detection using RAPTOR



Nanduri et al 2006. Sensors 6:808-822

Detection of cells with fiber optic sensor grown in BHI or EC at 2 h intervals



Geng et al 2006. Sensors 6:796-807

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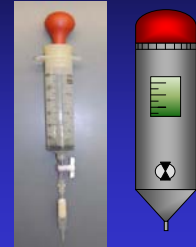
Summary: Fiber optic sensor for food pathogens

Pathogen	Detection limit	Detection time	Publications
<i>Listeria monocytogenes</i>	10 ³ cfu/ml 10 ⁴ -10 ⁵ cfu/ml meat	23 h	•Geng et al. 2004. AEM. 70: 6138 •Nanduri et al 2006. Sensors, 6:808 •Kim et al. 2006. Key Eng. Materials
<i>E. coli</i> O157: H7 SLT	10 ³ cfu/ml meat 0.5 ug/ml	4-6 h	•Geng et al. 2006. Sensors, 6:796 •Tu et al. 2006. SPIE
<i>Salmonella</i>	10 ⁴ cfu/ml	??	•Morgan et al. 2006. Key Eng. Materials

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Objective 3. Sample preparation strategy for use with microarray platforms

- Pathogen enrichment detection device (PEDD)
- Self-contained, easy-to-use device to deliver particle-free and pH adjusted bacterial suspension



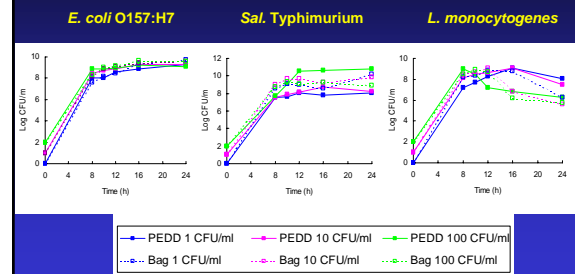
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Performance evaluation of PEDD with food sample

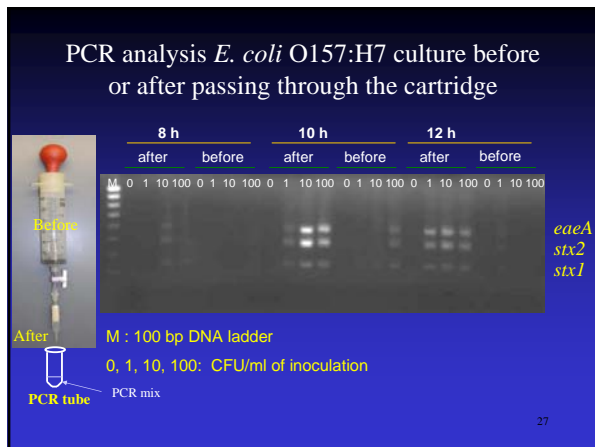
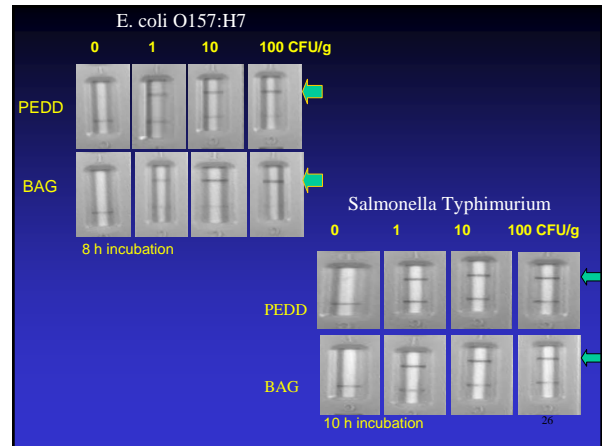
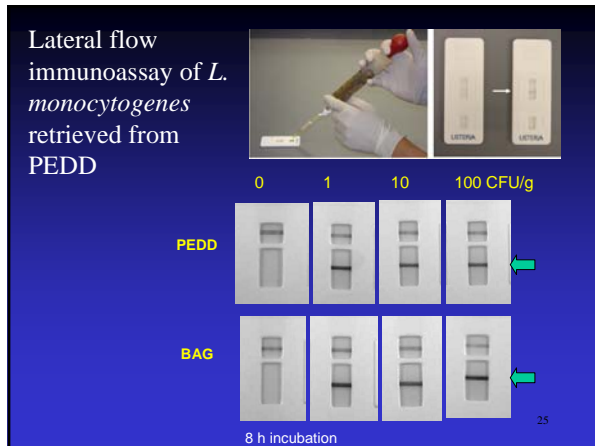
Pathogen	Food sample	Media	
		Growth	Detection
<i>E. coli</i> O157:H7	Ground beef	mEC + novobiocin	CT-SMAC
<i>Salmonella</i> Typhimurium	Egg	RV	XLD
<i>Listeria monocytogenes</i>	Hotdog	BLEB	MOX

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Growth of pathogens spiked onto food sample in PEDD



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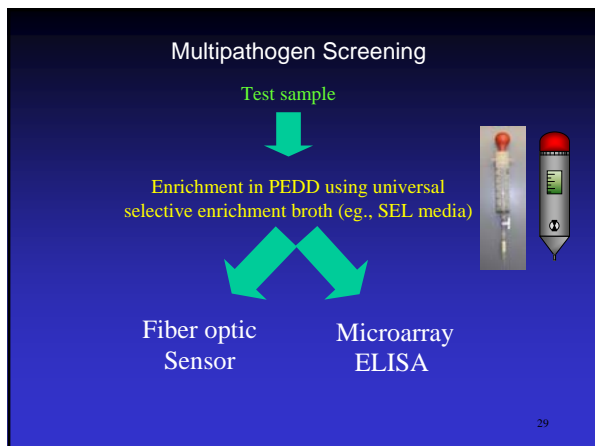


Objective 4: Formulation of selective universal enrichment broth for *Salmonella*, *E. coli* and *Listeria* (SEL)

- Media is called “SEL – *Salmonella*, *E. coli* and *Listeria*”
- Base – Buffered *Listeria* Enrichment Broth (BLEB) with acriflavin, cyclohexamide, fosfomycin, nalidixic acid)
- Media performance will be addressed in our next project.

Hyoehin Kim 2006

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Conclusions

- Specific antibodies are available for immunomicroarray assay
- Stress and media affect antibody reaction either by affecting transcription, translation or translocation
- Data with physiologically stressed bacterial cells would be helpful in formulating assay procedure for optimum signal
- Fiber optic sensor data are promising and multiplex assay could be developed using the automated system-RAPTOR
- PEDD system removes particles and inhibitors from food and should be useful for detection by biosensor platforms

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