Engineering of Biosystems for Detection of *Listeria monocytogenes* in Foods

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Outline

Introduction and Background
Microscale bio-separations: Cell Concentration and Recovery (CCR)
*L. Monocytogenes* microbiology for rapid probing and detection with CCR: PCR and optical detection
Nanotechnology and microfluidics for pathogen detection: Systems integration of biochip functions
Translational Activities
Next steps

Rapid Bacterial Detection Techniques

<table>
<thead>
<tr>
<th>Features</th>
<th>Growth Based</th>
<th>Viability Based</th>
<th>Cell Comp. Based</th>
<th>Nucleic Acid-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live/Dead</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Identification</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

- Need Growth Steps To Provide Live/Dead Information

We are growth-based and cell-component based approaches to provide Live/Dead and ID information

USDA Review, 2004

Benchmarks (Goals)

Concentrate sample containing bacteria
Final concentration of $10^3$ to $10^4$ cells / mL
Final viable cell count on chip > 10 cells
Concentrate cells in 30 min
Process samples in 60 min
Maintain cell viability
Introduce samples on chip, detect cells in 3 hr
Microscale bio-separations:
Cell Concentration and Recovery (CCR)

Conventional Methods
Conventional Sample Preparation Method
- Enrichment broth
- Oxford or LPM agars
- TSBYE agar
Commonly Used Detection
- ELISA-High detection limit
- Immunobeads-Small volume
- PCR-False-positive results

Bacterial growth during recovery:
function of nutrient availability

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S}
\]

Where
- \(\mu\) = growth rate (time\(^{-1}\))
- \(\mu_{\text{max}}\) = maximum growth rate (time\(^{-1}\))
- \(K_s\) = substrate concentration at \(\frac{1}{2}\mu_{\text{max}}\) (mass/vol)
- \(S\) = substrate concentration (mass/vol)

\(\mu \leq \mu_{\text{max}}\)

With excess substrate, \(K_s < S\)

\(\mu = \mu_{\text{max}} S < S\)

With very low substrate cells maintain viability, but are not growing

\[
\mu = \mu_{\text{max}} S < \frac{1}{2}\mu_{\text{max}} S
\]

Where \(k_e\) = maintenance constant

Poisson Distribution in presence of cell growth

gives estimate of minimum sample volume

\[
P(k) = e^{-\lambda} \frac{\lambda^k}{k!}
\]

where
- \(P\) = probability of capturing \(k\) cells
- \(C_0\) = cell concentration, cells / mL
- \(V\) = volume in mL to capture \(k\) cells
- \(T\) = processing time, min
- \(k\) = number of cells captured

Assumes
- (sample volume) \(\leq\) (total volume)/10
- medium is homogeneous; doubling time 30 min

Probability of capture of \(k\) (growing) cells in 50 \(\mu\)L
vs concentration of cells in sample, w/ cell growth

Probability of capture of \(k\) cells in 50 \(\mu\)L
vs concentration of cells in sample, no cell growth
Massaged Hotdog Experiment

CCR KiT Assembly
Uses membranes in series to process 100 mL hot dog extract into 0.1 to 1 mL sample

CCR Method

Membrane Properties
Careful selection of membranes
- Pore size and distribution
- Surface chemistry
- Interaction of target cell

Membrane Properties Image

Hollow Fiber Membranes

Hands-off System CCR
Current Status:
CCR delivers 10 cells onto biochip in 1 microliter if
Food extract volume is 100 mL containing 20 cells / mL
CCR concentrates food extract volume from
100 mL to 100 µL with 50% recovery of viable cells
CCR for a single, large volume better than replicates of
smaller portions of the same volume
Tests with L. monocytogenes, L. innocua, E. coli K12,
gfp E. coli, Pseudomonas and other microorganisms
Results given in posters presented in the laboratory area.

L. Monocytogenes microbiology for rapid
probing and detection of pathogens

General strategy for present work:
Recovery of live bacteria

Detection scheme

Recovery of Listeria with CCR kit

Validation with spiked hotdogs

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Fiber-optic signal acquisition for CCR kit recovered cells

Confirmation of L. monocytogenes by PCR

Fiber-optic detection of L. monocytogenes from mixed culture

PCR detection of L. monocytogenes from mixed culture

Assessment of expression of two virulence protein markers: ActA and InlB for potential use in immunosensor-based specific detection of L. monocytogenes
Low conductive medium for use with biochip

Medium with higher conductivity (>2000 µS) is not suitable for biochips.

High conductivity affects di-electrophoretic properties of bacteria thus hinders cell concentration by di-electrophoresis.

Decreased initial conductivity corresponds to decreased background noise.

LCGM—low conductive growth medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luria Bertani (LB)</td>
<td>Tryptone (5g/L)</td>
</tr>
<tr>
<td></td>
<td>Yeast extract (3 g/L)</td>
</tr>
<tr>
<td>LB with glucose (LBG)</td>
<td>Tryptone (5g/L)</td>
</tr>
<tr>
<td></td>
<td>Yeast extract (3 g/L)</td>
</tr>
<tr>
<td></td>
<td>Glucose (5g/L)</td>
</tr>
<tr>
<td>Low conductive growth</td>
<td>Tryptone, YE, Glucose, BSA</td>
</tr>
<tr>
<td>medium (LCGM)</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of *Listeria (Lm V7)* growth in various low and high conductive media

<table>
<thead>
<tr>
<th>Media</th>
<th>Initial conductivity</th>
<th>Lag period (h)</th>
<th>Generation time (h)</th>
<th>Highest OD 595</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>1200 µS</td>
<td>2-4</td>
<td>2.30</td>
<td>0.21</td>
</tr>
<tr>
<td>LCGM</td>
<td>1280 µS</td>
<td>2-3</td>
<td>1.00</td>
<td>0.67</td>
</tr>
<tr>
<td>TVC (Sy-lab)</td>
<td>2.8 mS</td>
<td>2</td>
<td>1.20</td>
<td>0.80</td>
</tr>
<tr>
<td>LRB Cellolosie</td>
<td>15 mS</td>
<td>&lt;2</td>
<td>0.50</td>
<td>1.45</td>
</tr>
<tr>
<td>LRB Glucose</td>
<td>15 mS</td>
<td>&lt;2</td>
<td>1.00</td>
<td>1.37</td>
</tr>
<tr>
<td>BLEB</td>
<td>16 mS</td>
<td>2</td>
<td>1.00</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Generation time (GT) = GT (OD2x) - GT (ODx).

Additional Experiments

- Growth kinetics of common and representative microorganisms from water, food, and clinical samples.
- Growth kinetics of stressed *Listeria* cells.
- Strategies to use LCGM with stressed cells.

Overview of Our Detection Process

- Concentrated Sample (cells) → Resuscitation in TSB for 2-4 h → Media exchange to LCGM.

Nanotechnology and microfluidics for pathogen detection

- Food Sample → Cell Extraction, Concentration, & Recovery → On chip: Concentration & Specific Capture → Detection of cell growth.

Total Time less than x hours!
**Integrated Systems for Study of Microorganisms and Cells**

- **Fluidic Ports**
  - On-chip Detection
  - Micro-scale Spectroscopy
  - Nanopores
  - Cantilevers
- **Cell Sorting**
- **Selective Capture**
- **Growth Detection**
- **DNA, protein Lysing**
- **Microscale Impedance Spectroscopy**
- **Nano-probe Array**

**“Lab on a Chip”**

**Cell Sorting and Concentration on a Chip: Dielectrophoresis**

- **Polystyrene beads**: \( \varepsilon_p < \varepsilon_m \rightarrow \text{negative DEP} \)
- **Cells**: \( \varepsilon_p < \varepsilon_m \rightarrow \text{negative DEP} \)
- **Cells**: \( \varepsilon_p > \varepsilon_m \rightarrow \text{positive DEP} \)

**Overall Detection Process**

- **Antibody to Listeria Monocytogenes**
  - 150kD IgG
  - Binds to a 66kD surface protein
  - Biotinylated in our lab

**Results - Incubation time: ~40 min**

- **Before rinse**
- **After rinse**

- ~8% captured
- ~100% gone

**Bacterial Growth & Detection**

- Invented in late 1800s (Petri and Koch)
- Still the most widespread means to grow and detect the presence of bacteria!

- **Doubling time ~ 20-40min**
- 1 \( \rightarrow \) 10^7 will take > 12 hours to reach
- Lag phase of few hours
- Total time to reach stationary phase > 20 hours
**Impedance Microbiology**

- Proposed in 1889
- Demonstrated first in 1912 by growth of the ‘typhus bacillus’
- Dead cells do not produce a signal

**Impedance Microbiology on a Chip**

- A large cell concentration can be achieved by confining a few bacteria in a small volume
- On-chip miniaturization → Short detection time
- Electrical detection (Impedance Microbiology) → Automation

**Micro-fabricated Biochip for Bacterial Cell Culture**

- DEP-based concentration system collects particles from a large flow stream and diverts them to a smaller stream

**Micro-fluidic Polymer Devices for Culture Bacteria and Spores**

- woo-Jin Chang, Demir Akin, Miroslav Sedlek, Michael Ladisch, Rashid Bashir, “Hybrid Poly(dimethylsiloxane) (PDMS)/Silicon Biochips For Bacterial Culture Applications”, Biomedical Microdevices 5:4, 281-290, 2003,

**Cell Concentration**

On-Chip Incubation of L. innocua

Detection of Cell Growth

Conclusions
Rapid cell concentration and recovery
Non selective adsorption minimized
Hands-off system being tested
Massaged Hot dog samples tested

Detection Microbiology
CCR validated using PCR, fiber optic probe
Special media and buffers identified
Microbial consortia developed for validation

Nanotechnology and microfluidics
On-chip systems achieve cell capture
Detection of cell growth validated
Polymer devices being developed

Next Steps
Cell Concentration and Recovery
Define bio-materials, mechanisms, models, configurations, and operating systems for homogenized meat and vegetable samples.

Pathogen detection microbiology
Identify mechanisms of pathogenesis, expression of pathogenic traits, and conditions for electronic detection of pathogenic cells

Nanotechnology and Microfluidics
Integration of systems for rapid detection and identification of viable cells using microfluidic devices.
Microfluidic modeling, design, and device fabrication