Rapid Separation and Concentration of Bacterial Pathogens in Foods

Wan-Tzu Chen $^{1,3}$, Rick Hendrickson $^{3}$, Tao Geng $^{2}$, Arun Bhunia $^{2}$, Michael R. Ladisch $^{1,3,4}$

$^{1}$Department of Biomedical Engineering  
$^{2}$Department of Food Science  
$^{3}$Laboratory of Renewable Resources Engineering  
Integrative Center of Biotechnology and Engineering  
$^{4}$Department of Agriculture and Biological Engineering  
Purdue University
Acknowledgement

This research was supported through a cooperative agreement with the ARS of the United States Department of Agriculture project number 1935-42000-035

- Dr. Richard Linton (FSEC at Purdue University)
- Dr. Rashid Bashir
- Debby Sherman and Chia-Ping Huang
- LORRE group
Outline

- Introduction
- Objectives
- Materials and Methods
- Results
- Conclusions
Introduction

Foodborne Pathogens
- Cause diseases associated with food
- Millions of people get ill annually
- Food samples are complex with interfering substances
- Conventional detection techniques are too costly and time-consuming
Introduction

- *Listeria monocytogenes*
  - Gram-positive, rod-shaped bacterium
  - Highly acid/salt-resistant
  - Cause listeriosis
    - Average death rate of 20~30%
    - Especially harmful for pregnant women
  - Occur in milk, cheese and ready-to-eat dairy food via post-processing contamination
Overall Objective

A rapid detection procedure based on a silicon-based chip system
Biochip Project

- Our approach:
  
  Sample preparation from foods. Concentrate bacteria for 10000x

- Conventional approach:
  
  Up to a week for identification

Timely result with Biochip within several hours
Research Goals

- Separate target microorganisms from interfering substances like proteins and lipid
- Concentrate by 10000x in 10 min to avoid time-consuming culture step of 24 hours
- Keep target microorganisms alive for identification
- Recover microorganisms efficiently
Materials and Methods

- Membrane filtration of spiked samples
- Hotdog juice preparation
- Efficiency of recovering and concentrating target microorganisms
Membrane Filtration

- Widely used in separation, concentration and recovery of biomolecules
- Simplify the procedures of diluting and concentrating
- Surfactant/Enzyme proved to improve filtration rate

Peterkin et al
~700 cells/ml × 50 ml

Assumption: 1 mg = 1 µl

Each membrane contains ~15 µl of liquid

By membrane filtration, $10^4$ cells can be concentrated inside 15 µl of liquid
How do we get them all off?
Surfactant Effects

- Non-ionic surfactant
- Reduce the surface tension of liquid
- Prevent *Listeria* from sticking on the membrane
Inoculated hotdog juice
700 cells/ml

Different volumes are filtered with syringe

Membrane filter

Membrane filters are immersing in 0.5 ml of PBS containing 1% Tween 20

Filtrate

Plate out on MOX agar

Bradford protein assay
Hotdog Juice Preparation

• 1 pack of hotdog immerse into 250 ml of PBS (pH 7.4)
• Use stomacher bag to massage the hotdogs
• Incubate for 2 hours
• Filter unit with 0.2 µm pores of cellulose nitrate (Sterilization)
## Membrane Filters

<table>
<thead>
<tr>
<th></th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nylon</td>
<td>-(CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CONH-)$_n$-</td>
</tr>
<tr>
<td>PVDF</td>
<td>-(CH$_2$-CF$_2$-)$_n$-</td>
</tr>
<tr>
<td>Mixed Cellulose</td>
<td>Cellulose nitrate and cellulose acetate</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>-(O- O-C(CH$_3$)$_2$-O- CO-)$_n$-</td>
</tr>
</tbody>
</table>
Results

- Preliminary tests
- Bradford Protein Assay of filtrates
- *Listeria* concentration calculated from plating-out
Plate count for 0.45\(\mu\text{m}\) retentate

Theoretical number of bacteria
- Polycarbonate
- Nylon
- Mixed Cellulose
- PVDF
Membrane Filters Properties

Nylon membrane

PVDF membrane
Membrane Properties

Polycarbonate membrane
Permeate Assay

Protein Conc (mg/ml)

Polycarbonate 0.4 um
Mixed Cellulose 0.45 um
Control-blank Hotdog juice

Filtered volumes (ml)

10 ml
50 ml
## Tween 20 Effect

<table>
<thead>
<tr>
<th>PC</th>
<th>Recovery percentage (%)</th>
<th>Mixed Cellulose</th>
<th>Recovery percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 um</td>
<td></td>
<td>0.45 um</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>5.27</td>
<td>1 ml</td>
<td>3.44</td>
</tr>
<tr>
<td>Tween</td>
<td>65.36</td>
<td></td>
<td>70.41</td>
</tr>
<tr>
<td>1 ml</td>
<td></td>
<td>5 ml</td>
<td>5.05</td>
</tr>
<tr>
<td></td>
<td>5.27</td>
<td></td>
<td>86.28</td>
</tr>
<tr>
<td>5 ml</td>
<td></td>
<td>5 ml</td>
<td>5.05</td>
</tr>
<tr>
<td></td>
<td>5.27</td>
<td></td>
<td>86.28</td>
</tr>
<tr>
<td>10 ml</td>
<td>8.44</td>
<td>10 ml</td>
<td>4.61</td>
</tr>
<tr>
<td></td>
<td>67.68</td>
<td></td>
<td>38.03</td>
</tr>
<tr>
<td>25 ml</td>
<td>8.99</td>
<td>25 ml</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>48.35</td>
<td></td>
<td>13.94</td>
</tr>
<tr>
<td>50 ml</td>
<td>10.78</td>
<td>50 ml</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>72.24</td>
<td></td>
<td>58.48</td>
</tr>
</tbody>
</table>
Concentration Factor

- Expressed by concentration ratio

\[
\frac{\text{Listeria concentration after filtration (cells/ml)}}{\text{Listeria concentration before filtration (cells/ml)}}
\]
How Concentrated Are They?

Concentration factors

Filtered volumes (ml)

- Polycarbonate 0.4 um
- Mixed cellulose 0.45 um
- Expected concentrated factors
Pore Sizes Effects

Filtered volumes (ml)

- Polycarbonate 0.4 um
- Polycarbonate 0.2 um
Pore Sizes Effects (cont.)

- **Mixed cellulose 0.45 um**
- **Mixed cellulose 0.22 um**

Filtered volumes (ml):
- 1 mL
- 5 mL
- 10 mL
- 25 mL
- 50 mL

Concentration factors
Conclusions

- Membrane filtration can easily concentrate *Listeria monocytogenes* into small volumes and separate interfering substances from microorganisms
- Tween 20 treatment facilitates *Listeria* recovery
- Recovery increases as pore sizes decrease
- Time taken is only 2 hours compared to traditional culture steps
Questions?