Biofunctional membranes for *Listeria monocytogenes* detection

Wan-Tzu Chen \(^1,2\), Michael R. Ladisch \(^1,2,4\), Tao Geng \(^3\), and Arun K. Bhunia \(^3\)

\(^1\) Department of Biomedical Engineering, Purdue University
\(^2\) Laboratory of Renewable Resources Engineering, Purdue University
\(^3\) Department of Food Science, Purdue University
\(^4\) Department of Agricultural and Biological Engineering
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Foodborne Pathogens

✓ Bacteria, Virus and Parasite

✓ Potential Hazardous Food
  ➢ Temperature, acidity, time, oxygen…..etc

✓ Syndromes
  ➢ Abdominal pains, vertigo, diarrhea, intoxication…..etc

✓ Examples
  ➢ C. botulinim, Salmonella, L. monocytogenes
Listeria monocytogenes

✓ Gram-positive, rod-shaped bacterium
  ➢ Highly resistant to salt and low temperature

✓ Conventional Sample Preparation Method (BAM, 1998)
  ➢ Enrichment broth
  ➢ Oxford or LPM agars
  ➢ TSBYE agar

✓ Rapid Sample Preparation Method
  ➢ Membrane filtration
  ➢ Decreased culture time
Syringe Filter System

Inoculated hotdog meat broth (HDM) ~700 cells/ml

Membrane immersing in 0.5 ml of PBS with 1% of Tween 20 for 30 minutes

Different volumes being filtered

Membrane filters

PVDF
Nylon
Mixed cellulose
Polycarbonate
In 0.45 um and 0.22 um nominal size

Filtrates

Plate out 100 µl on MOX agar

Bradford Protein Assay
Tween 20

- Non-ionic surfactant
- Produce surface liquid tension
- Prevent protein binding
- Hence help *Listeria* recovery
0.45 um with Tween 20

![Graph showing concentration factors for filtered volumes of 1 mL, 5 mL, 10 mL, 25 mL, and 50 mL using Polycarbonate 0.4 um, Mixed cellulose 0.45 um, and Theoretic maximal concentration factors.]
Smaller Pore Sizes

Filtered volumes (ml)

Concentrated factors

Nucleopore 0.2 um
Nucleopore 0.4 um
MicronSep 0.22 um
MicronSep 0.45 um
Different Pore Structures

Mixed Cellulose (Depth filter)  Polycarbonate (Screen filter)

Listeria monocytogenes

Ladisch, 2001
More Dilute Samples (15 cells/ml)

Recovery rate~70% = 37 cells/ 20 ul
Volume below 0.13 ml results in no bacteria recovered

$y = 10.352x - 1.2721$

$R^2 = 0.9863$
New Thoughts

Bacteria
Antibodies
Membrane
Chemistry of Antibodies

Immobilization


Enzyme

Antibody

P66
Antibody Immobilization

Membrane+Poly-Lysine+Glutaraldehyde (LG surface)  
LG surface + FITC-P66
Listeria on Different Surfaces

5*10^7 cells/ml

Listeria on LG surface—HIGH non-specific binding!

Listeria on BSA surface—blocking non-specific binding
Specific Binding

Initial = $5 \times 10^7$ cells/ml

Blank LG surface

Listeria on P66 surfaces
Non-specific *E. coli* Binding

Initial = $5 \times 10^7$ cells/ml

Blank LG surface

*E. Coli* on P66 surfaces
Conclusions

- Membrane filtration helps separation
- Tween 20 treatment facilitates *Listeria* recovery
- Recovery increases as pore sizes decrease
- Antibody immobilization works well on PC
- Bacteria differentiation can be carried out