Bioseparations for Biochips

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Introduction

- Foodborne pathogens
  - Pathogens associated with food
  - Cause huge social capital annually
  - Time-consuming procedures for conventional techniques
  - Need faster and more accurate detection
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
Rationale

- Food proteins might block the binding sites on the chips and prevent accurate binding of *Listeria* to antibody.

- To insure the samples flowing through chips contain only microorganisms.

- Target organism needs to be concentrated to enhance detection sensitivity.
Main Research Objectives

Use chromatographic resins for micro-scale separation

- Remove proteins and clean the sample broth
- Concentrate target microorganisms
Reverse-Phase Chromatography

- Common analytical method for protein analysis.
- Hydrophobic hydrocarbon chains on silica beads.
- Components elute out in the decreasing order of polarity.
- Gives fingerprint of proteins in sample
Hotdog Meat (HDM) Broth

- Blend the hotdogs with PBS buffer (pH 7.4)
- Centrifuge
- Filter
- Analyze the protein contents
Fingerprint of Protein Chromatogram

Auto-Scale Chromatogram

Minutes
Cation vs. Anion Exchange Adsorbents

- **Cation ion exchanger**
  - Negatively charged surface to grab positively charged proteins.

- **Anion ion exchanger**
  - Positively charged surface to grab negatively charged proteins
List of Resins Examined in this Work

<table>
<thead>
<tr>
<th>Cationic ion exchanger</th>
<th>Anionic ion exchanger</th>
<th>Hydrophobic Resin</th>
<th>Bifunctional Resin</th>
<th>Reversed-Phase Resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>- IRA 120+</td>
<td>- DEAE cellulose</td>
<td>- Butyl 650S</td>
<td>- Amberlite IRN 150</td>
<td>Amberlite XAD2</td>
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<tr>
<td>- Amberlyst 35</td>
<td>- DEAE 650M</td>
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<td>- Hydroxylapatite</td>
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<tr>
<td>- SP 550C</td>
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<td></td>
<td>- Super 650M</td>
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<td>- IRA 400</td>
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<tr>
<td></td>
<td>- Silica</td>
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</table>
Protein Removal

- Mix resin with HDM broth
- Incubate at pH 7.4 for several time intervals
- Filter the samples
- Analyze protein concentration
  - Bradford protein assay
  - Reverse-phase chromatography
Protein Removal-Protein Assay

Protein remaining%

Amberlyst 35 (strong cation) DEAE cellulose (weak anion)

Resins

0 min 30 min 90 min
Strong and Weak Ion Exchanger

- **pH effect on ion exchanger capacity**

  - **Cation**
  - **Anion**

  - Strong cationic exchanger
  - Weak cationic exchanger
  - Strong anionic exchanger
  - Weak anionic exchanger

  - pH = 7.4

Ladisch, 2001
What’s next?

- Non-specific protein removal achieved by chromatographic resins
- Specific *L. monocytogenes* capture by smaller spheres
Specific *L. monocytogenes* Capture by Immunomagnetic Separation (IMS)
Immunomagnetic Separation

Streptavidin-coated beads (2.8 µm) → Biotin-Ab → Listeria monocytogenes

Streptavidin

Biotin-Ab

Bugs
ELISA test of binding chemistry

Substrate (OPD) -> Color change

- ExtrAvidin-Peroxidase
- Biotin
- Protein A
- C11E9 or P66

Antigen on plate (surface proteins)
Results of ELISA test

- Ag+Biotin-proteinA-P66
- Ag+Biotin-proteinA-C11E9
- Ag+biontin-P66
- Ag+Biotin-C11E9
- Ag+Extravidin-peroxidase
- P66+Ag
- C11E9+Ag

Abs 490 nm
Fluorescence test

- Use of fluorometer to differentiate layers
- Procedures:
  - Beads + biotin-Protein A + C11E9 or P66
  - Use of FITC labeled Anti-mouse IgG to verify the binding
Results of Fluorescence test

- Beads+Biotin-Protein A+P66+FITC Anti-mouse IgG
- Beads+Biotin-Protein A+C11E9+FITC Anti-mouse IgG
- Empty beads+P66
- Empty beads+C11E9

AU
From ELISA and Fluorescence tests

- Protein A helps the chemistry of binding
- Polyclonal antibody is more active than monoclonal antibody
- Need to test *Listeria* capture
### L. monocytogenes capture

<table>
<thead>
<tr>
<th>Inoculum level (cfu/ml)</th>
<th>C11 E9 capture (cfu/200 µl)</th>
<th>P66 capture (cfu/200 µl)</th>
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<tbody>
<tr>
<td>203</td>
<td>11</td>
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<td>2030000</td>
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Conclusion

- Most protein uptake by strong cationic exchanger suggests that HDM might contain more positive charged proteins.
- Protein A enhance the binding of antibody to beads.
- *Listeria monocytogenes* capture as low as 200 cells/ml can be achieved.
- IMS can also concentrate the pathogens to small volumes.
Protein Removal by Resins
Protein Removal-RPC

Protein remaining (%)

Resins

Amberlyst 35 (strong cation)

DEAE cellulose (weak anion)

0 min

30 min

90 min
Conclusions - protein removal

- Different resins adsorb proteins from HDM broth
- Most protein uptake by strong cationic exchanger suggests that HDM might contain more positive charged proteins
- Food protein removal is crucial in foodborne pathogen detection
Comparison of lightness

Empty beads

Beads+FITC-Ab

Beads+biotin-C11E9

Beads+biotin-C11E9+FITC-Ab