Infrared Sensors for Rapid Identification of Select Microbial Foodborne Contaminants

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Project Milestones

1. Create a library of FT-IR spectra of bacterial cell wall components and whole cells (from *Salmonella*, *Campylobacter jejuni*, and *Escherichia coli* O157:H7) needed for cell identification and differentiation.

2. Develop FT-IR methods for cell identification and quantification in water, cultural media, and foods.

3. Develop a limited wavelength approach for cell identification.

4. Build and validate an IR sensor based on the most promising few-wavelength algorithm developed using FT-IR techniques developed in the first two milestones.
FT-IR instrument

ThermoElectron, Nexus 670
Milestone 1: Library of cell wall components and whole cells

Progress: *E. coli* and *Salmonella*
Major Bacterial Mid-IR Peaks

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3500</td>
<td>OH stretching</td>
</tr>
<tr>
<td>2</td>
<td>3200</td>
<td>NH stretching (amide A) of proteins</td>
</tr>
<tr>
<td>3</td>
<td>2959-2852</td>
<td>CH, CH₂, CH₃ stretching in fatty acids</td>
</tr>
<tr>
<td>4</td>
<td>1655-1637</td>
<td>Amide I of α-helical &amp; β-pleated structures</td>
</tr>
<tr>
<td>5</td>
<td>1548</td>
<td>Amide II band</td>
</tr>
<tr>
<td>6</td>
<td>1515</td>
<td>Tyrosine band</td>
</tr>
<tr>
<td>7</td>
<td>1468</td>
<td>CH₂ bending of methylene</td>
</tr>
<tr>
<td>8</td>
<td>1310-1240</td>
<td>Amide III band components of proteins</td>
</tr>
<tr>
<td>9</td>
<td>1250-1220, 1084-1088</td>
<td>PO2 stretching of phosphodiesters</td>
</tr>
<tr>
<td>10</td>
<td>1200-900</td>
<td>C-O-C, C-O of various polysaccharides</td>
</tr>
<tr>
<td>11</td>
<td>720</td>
<td>CH₂ rocking of methylene</td>
</tr>
<tr>
<td>12</td>
<td>900-600</td>
<td>&quot;Fingerprint Region&quot;</td>
</tr>
</tbody>
</table>

(Filtered *Salmonella Typhimurium* spectrum)
Select Bacteria

- **Escherichia coli**
  - *E. coli* DH5α, *E. coli* K12, *E. coli* O157:H7, *E. coli* O157:12, *E. coli* O157:19

- **Salmonella**
  - *Salmonella enterica* serovars
    - S. Typhimurium, S. Enteritidis, S. Thomasville, S. Brandenburg, S. Hadar, S. Seftenberg
  - ~250 LPS isolates from J. Bouldin (USDA-ARS)

- **Campylobacter**
  - C. jejuni, C. lari, C. coli
Lipopolysaccharides (LPS)

• Lipid A
  - Highly conserved

• Core oligosaccharides (Core OS)
  - Non-repeating sugars
  - Inner/Outer

• O-polysaccharides (O-PS)
  - Repeating unit: 3-5 sugars
  - High variation
DOC-PAGE (*Salmonella* LPS Extracts)
FTIR Spectra of *Salmonella* LPS Extracts

- **S. Typhimurium**
- **S. Enteritidis**
- **S. Thomasville**
- **S. Brandenburg**
- **S. Hadar**
- **S. Seftenberg**
CVA-PCA (Salmonella LPS Extracts)
FTIR Spectra of intact *Salmonella* cells
CVA-PCA (intact *Salmonella* cells)
Summary of Results

• FTIR spectra of crude LPS extracts in combination with chemometrics successfully differentiated and classified between *E. coli* strains and between *Salmonella* serotypes.

• Carbohydrate region (1,200-900 cm\(^{-1}\)) was better suited for spectral analysis of crude bacterial LPS extracts than the entire mid-IR region (4,000-700 cm\(^{-1}\)).

• FTIR spectra of intact cells of *Salmonella* serotypes failed to differentiate between *Salmonella* serotypes, with the exception of *S. Brandenburg* due in part to specific peaks in the amide region (1,800-1,500 cm\(^{-1}\)).

• FTIR spectra of crude bacterial LPS extracts may facilitate taxonomical or epidemiological studies of microorganisms in a rapid, sensitive, and accurate way.
Milestone 2: FT-IR method development for cell identification and quantification in water, cultural media, and foods

Progress: *E. coli* and *Salmonella*
Detection of **whole cells** of
*Salmonella* Typhimurium and *E. coli*
O157:H7
IR Microscope Method

Membrane filtration

Contact ATR

Filtration Apparatus
Membrane Filter
Vacuum

Contact ATR Objective
Pressure Sensitive Stage
Membrane Filter
Contact Indicator Panel
Summary of Results

• Filtration of bacterial samples is suitable for sample preparation and analysis using the contact ATR accessory of the FT-IR Continuum Microscope.

• The sensitivity of detection using the FT-IR was 500 CFU/mL following incubation of the Salmonella Typhimurium/ E. coli O157:H7 in TSB for 6 h.

• This FT-IR method required less time and is less expensive than previous FT-IR methods.
Detection of *E. coli* O157:H7 in Fruit Juices
Main Bench ATR Method

• Samples concentrated on Metricel® Filter using the filtration apparatus

• Spectral Collection (256 scans, 4 cm^{-1}): FTIR main bench using ZnSe Flat Plate ATR accessory and Pressure Clamp

• Discriminant Analysis (DA): TQ Analyst to discriminate between *E. coli* O157:H7 and *E. coli* K12 in the amide II region
Summary of Results

• DA was able to classify the pathogenic *E. coli* O157:H7 from the non-pathogenic *E. coli* K-12 in all fruit juices analyzed without any misclassification.

• Background matrix (juice type) did not significantly impact the analysis of the spectrum.
Selectivity experiments

• To improve specificity of detection of *E. coli* O157:H7 by use of selective enrichment
Summary of Results

• The selective enrichment of modified EC broth is effective against non-targets such as *Pseudomonas aeruginosa* and *E. coli* O157:H7 is now detectable at the 6th dilution upon incubation.

• The sensitivity of detection using the FT-IR was 500 CFU/mL following selective enrichment and 7 h incubation of the *E. coli* O157:H7
Summary of Results

• Use of *E. coli* O157:H7 specific Dynabeads® to capture *E. coli* O157:H7 in a mixed broth system works

• Discriminant analysis was able to classify the pathogenic *E. coli* O157:H7 from the non-pathogenic *E. coli* K-12 without any misclassifications

• These findings can be further extended to liquid food systems and mixed culture systems
Milestone 3: Develop a limited wavelength approach for cell identification
Algorithm

\[
C = c_1 + c_2 \frac{IA_{\lambda,B1}}{IA_{\lambda,bl}} + c_3 \frac{IAR_{\lambda,B2}}{IA_{\lambda,bl}}
\]

Where:
- \( C \) = Bacterial concentration
- \( c_i \) = Calibration constants
- \( IA_{\lambda,B1} \) = Integrated absorbance (sensor signal) for first bacterial absorbance region
- \( IA_{\lambda,B2} \) = Integrated absorbance (sensor signal) for second bacterial (or interference) absorbance region
- \( IA_{\lambda,bl} \) = Integrated absorbance (sensor signal) for baseline absorbance region
Milestone 4: Build and validate an IR sensor based on the most promising few-wavelength algorithm
ATR MicrobeSensor

Pulsable IR Source with reflector

Quad detector with filters

Source modulator

Signal conditioning and calibration

Sample

ATR Crystal
Presentations


Questions?