Rapid and Specific Detection of STEC Strains O157:H7, O26 and O111 Using a Label-Free Biosensor System

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Introduction

Pathogens

- *Escherichia coli* O157:H7, a strain of enterohemorrhagic, Shiga toxin-producing *E. coli* (STEC), causes gastroenteritis, resulting in (bloody) diarrhea and sometimes acute kidney failure due to HUS (hemolytic-uremic syndrome).
- *E. coli* O26 and O111 are STEC strains, similar to O157:H7, and are emerging threats to human health.
- *Salmonella enterica* serovar Typhimurium causes gastroenteritis, resulting in diarrhea, fever and abdominal cramping.

Incidence

- Annually in the U.S. there are >100,000 cases of STEC and >1.4 million non-typhoidal *Salmonella* infections.

Problem

- Detection of foodborne pathogens is the primary means of preventing contaminated foodstuffs from entering the market.
- Traditional immunoassays are the gold standard for identifying specific strains of bacteria. However, while specific, these immunoassays are time-consuming.
- Most current biosensor applications focus on specificity or sensitivity rather than assay speed.

Materials and Methods

Antibodies

- Polyclonal antibodies against *E. coli* O157 (KPL Cat. # 01-95-90), *E. coli* O26 (KPL Cat. # 01-95-92), *E. coli* O111 (KPL Cat. # 01-91-99) and *Salmonella* species (KPL Cat. # 01-91-99) were used as capture antibodies.

Materials

- Bacteria were serially diluted in EDTA buffer (10 mM phosphate, 150 mM NaCl, 10 mM EDTA).
- Bacteria were disrupted by sonication and 1% Triton X-100 prior to pelleting; supernatants were assayed.
- Human serum was diluted 1:10 in EDTA buffer before use.
- Hamburger (25 g/225 mL buffer) was sonicated, pelleted and filter sterilized before use.

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Model

- Biosensor: streptavidin functionalized.

Results – Disrupted Bacteria

- Time-consuming enrichment steps were avoided.
- Processing of the samples, in order to make a more uniform layer on the biosensor, was needed for higher levels of sensitivity.

Results – Beef Extract

- Detection limits of *E. coli* O157 in hamburger extract were achieved with cognate biotinylated polyclonal antibodies.

Results – Human Serum

- Detection limits of *E. coli* O157 in human serum were achieved with cognate biotinylated polyclonal antibodies.

Summary

- Detection of bacterial pathogens has been demonstrated in multiple matrices, for the first time, using the commercially available Octet Red96 biosensor from ForteBio.
- Biolayer interferometry, in combination with KPL’s polyclonal BacTrace® antibodies, is a viable method for rapid, specific detection of various bacterial strains, including the pathogens of *E. coli* O157, *E. coli* O26, *E. coli* O111 and *Salmonella* Typhimurium.
- Using BLI, as few as 5 x 10⁵ CFU/mL of bacteria can be detected in less than four hours (total assay time).
- Time consuming enrichment steps were avoided.
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