# Characterization and Validation of Phage Oligopeptide Probes for Detection of *Salmonella enterica* serotypes using a Magnetoelastic Sensor

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*Salmonella enterica* is commonly associated with food poisoning in countries all over the world. This species has approximately 2500 serovars that are divided into four different O-antigen groups. A rapid sensor test for all relevant ones is desirable to improve domestic food safety procedures. In our research, we used phage display technology combined with lipopolysaccharide (LPS) antigens extracted from bacterial cell surfaces of different groups of serovars to develop phage probes that bind with *Salmonella enterica* serotypes, and demonstrated the use of the phage on rapid magnetoelastic biosensor systems as a front-line detection ligand.

We modified a phenol-chloroform-petroleum ether (PCP) extraction method to maximize the extraction of LPS from cell surface of seventeen representative foodborne *Salmonella enterica* serovars in O-antigen B, C, and D groups. Group A was not included since it contains the typhoid serotypes. The purified LPS was used in lieu of cultures to screen for phage peptides through a Phage Display method. Binding with the selected phages was validated using a phage capture ELISA procedure and a whole cell ELISA technique.

Six selected phage peptides all demonstrated high affinity in ELISA to isolated LPS antigens (5-10 fold higher) and to *Salmonella* whole cell mixture (3-5 fold higher), when compared to control phage M13 (Fig.1). Phage C4-22 was selected as the best candidate for use on a sensor since it was consistently specific with a high level of affinity to *Salmonella* whole cells and low binding to other related Enterobacteriacae members such as *Shigella* and *Escherichia coli* O157:H7 (Fig.2).

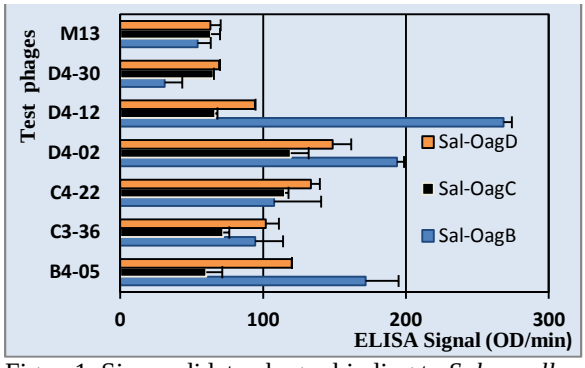


Figure1. Six candidate phages binding to *Salmonella* whole cells using the ELISA test.

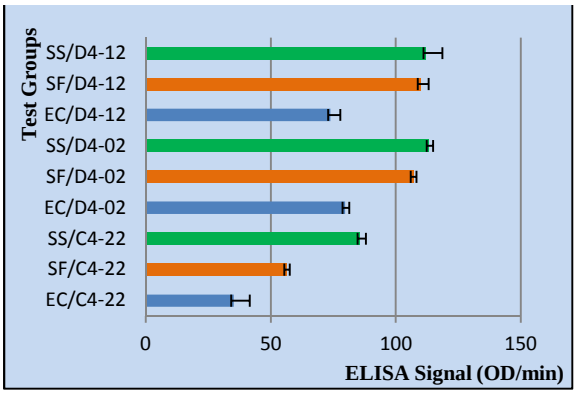


Figure2. Binding of candidate phages to *Shigella sonneii* (green bars), *Shigella flexneri* (red bars), and *E. coli* O157:H7 (blue bars) in a whole cell ELISA test.

In the magnetoelastic biosensor testing system, *Salmonella typhimurium* solutions at three concentrations of $5x10^7$, $1x10^8$, and $5x10^8$ cfu/ml were reacted to phage coated sensors. BSA (0.1%) was used as a blocking reagent. Sensors coated with 0.1% BSA only served as controls. Cells of *Salmonella typhimurium* detected on the sensor were washed with TBS/0.5% Tween three times and eluted with 0.1M Glycine to break phage-*Salmonella* binding. The percent of *Salmonella* binding to the phage coated sensor was determined using a standard aerobic plate count method (APC). Phage C4-22 coated sensors demonstrated 30 times higher *Salmonella* binding capacity than the control sensors at the *Salmonella* loading concentration of $5x10^8$ cfu/ml (Fig.3).

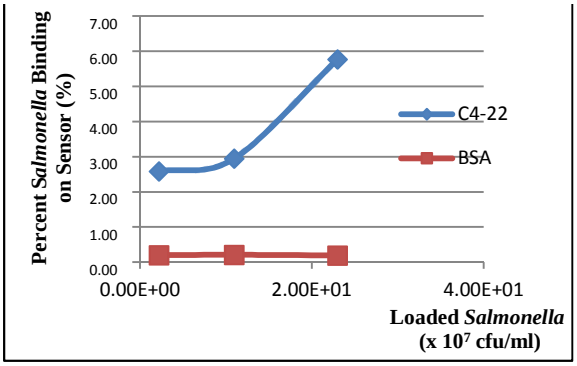


Figure3. Comparison of percent *Salmonella* binding on phage and non-phage sensors.

The modified PCP LPS extraction method enabled the use of these tested *Salmonella enterica* serotypes antigens to produce peptides that bind with the cell surface of all representative *Salmonella* (O-antigen groups B, C, and D) tested to date. The phage C4-22 is a strong candidate for use with rapid sensor testing platforms for *Salmonella* detection in foods.

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