Abstract #2824, 224th ECS Meeting, © 2013 The Electrochemical Society

Strategies for isolation of phage displayed oligopeptide probes for rapid detection of pathogenic *Salmonellae*

Zhou Tong[1], Laura Silo-Suh[2], and Sang-Jin Suh[1]

[1]Department of Biological Sciences, 101 Life Sciences Building, Auburn University, Auburn, AL 36849
[2]Department of Basic Medical Sciences, 1550 College St., Mercer University School of Medicine, Macon, GA 31207

Pathogenic serovars of *Salmonella enterica* pose serious public health concern as foodborne and zoonotic pathogens. This fact was emphasized by several recent outbreaks of *S. enterica* in United States. From eggs to poultry, from peanut butter to fresh produce such as tomatoes and cucumbers, and from hedgehogs to small turtles, pathogenic *S. enterica* is transmitted to humans in various ways. In order to prevent an outbreak it is imperative to develop an accurate, fast, sensitive, cost effective, and user-friendly detection mechanism. Such a detection mechanism can be used as the first screen to ascertain the presence or absence of pathogenic *Salmonellae* so that appropriate therapeutic and preventive measures can be implemented.

Biosensor technology combines the accuracy and sensitivity of other approaches with improvement in rapidity of detection. A typical biosensor is composed of molecular probes immobilized on a platform with characteristics that can be easily measured based on the probe:analyte interactions. Recent development of biosensors composed of magnetoelastic particle and phage-displayed oligopeptides show enormous promise for their sensitivity, low cost, and user-friendliness. However, for the magnetoelastic biosensors to function properly, it is essential for them to be coupled with molecular probes that are highly specific for the analyte of interest. Phage-displayed oligopeptides are attractive as probe molecules because they are highly stable and resistant to environmental stressors while maintaining the ability to interact specifically with analytes.

In order to quickly diagnose and to prevent potential outbreaks of *Salmonella*, we sought to develop biosensors that can detect multiple serovars of that pathogen. Since the magnetoelastic platform has already been demonstrated to be highly effective, we focused on the isolation of molecular probes that can recognize multiple serovars of *Salmonella enterica*. Although over 2500 serovars of *S. enterica* have been described, only a few consistently cause disease in humans. We focused our attention on 17 non-typhoid *S. enterica* serovars that have recently caused outbreaks: *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, *S.* Javiana, *S.* Heidelburg, *S.* Montevideo, *S.* Branderup, *S.* Thompson, *S.* Infantis, *S.* Dublin, *S,* Mission, *S.* Derby, *S.* Panama, *S.* Diarizonae, *S. bungori*, *S.* Indica, and *S*. Salamae.

We took three different approaches to isolate phage-displayed oligopeptide probes that can recognize all seventeen pathogenic serovars of *S. enterica* used in our study. The Ph.D.-12 Phage Display Peptide library was purchased from the New England Biolabs (Beverly, MA). Prior to biopanning for *Salmonellae* specific peptide probes, the phage library was negatively selected against plastic, BSA, *Escherichia coli* O157:H7, *Shigella sonnei,* and *S. flexneri* to minimize isolation of nonspecific probes. In our first approach, we combined all seventeen pathogenic serovars of *S. enterica* in our collection and used the mixture as the whole cell analyte. We isolated several dozens of potential peptide probes from this approach. Unfortunately, upon more careful analysis, we failed to discover a subset of phage probes that recognized all seventeen serovars. We believed the problem with the first approach was the presence of too many potential binding sites on each bacterial cell that complicated the isolation of peptides that can recognize universal analytes present on all seventeen serovars. Our second approach was to streamline the seventeen serovars and perform sequential biopannings to enrich for those peptides that recognize putative universal cell envelope component on all seventeen serovars of *S. enterica*. Thus, we divided seventeen serovars into six groups based on the frequency of outbreaks caused by each organism between the years 2009 to 2012. In this approach, the phage library was initially biopanned twice against each group. The resulting phage were then pooled into one library that contained peptides that recognized organisms in each of the six groups. This library was next biopanned against each member of the group in a parallel fashion as shown in Figure 1. After three rounds of parallel biopanning, we isolated 61 peptide probes that appeared to recognize the top ten pathogenic serovars well. We determined the sequence of these probes and found 24 genotypes among the 61 sequences. Using the neighbor-joining approach, we divided the 24 amino acid sequences into 3 clusters. Cluster 1 had 17 probes with the sequence NSFWANWEHLWS, 14 probes with the sequence VHNTMWWGRQQP, and three others presented only once. Cluster 2 had five probes that appeared only once. Cluster 3 had 14 sequences of which one sequence appeared five times, two other sequences appeared three times, and 11 sequences that appeared only once. From this analysis, we isolated several phage probes that appear to be promising for detection of pathogenic *S. enterica*. In our third approach, we targeted a specific outer membrane protein (OMP) T2544 of *S. enterica*. We cloned the gene encoding for this OMP into the plasmid vector pQE80-L to express it in *E. coli*. We are currently in the process of isolating phage displayed peptide probes that specifically recognize this *S. enterica* OMP.

In this study, we devised and executed several strategies to isolate phage displayed oligopeptide probes that can be used to detect various pathogenic serovars of *S. enterica*. We are currently in the process of further characterizing our probes to determine their potential limitations and to further improve their selectivity.