Detection of *Edwardsiella tarda* by fluorometric and biosensor methods using a peptide ligand identified from a phage-peptide library

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*Edwardsiella tarda* (*E. tarda*), an enteric Gram-negative bacterium, is known as a causative agent of edwardsiellosis in many fishes. The purpose of this work is to identify a peptide ligand from a phage-peptide library for detection of *Edwardsiella tarda* (*E. tarda*). After biopanning of the library against *E. tarda*, a peptide was selected and synthesized with a fluorescent label. The fluorescent peptide was used for detection of *E. tarda* by the conventional plate assay method. When *E. tarda* was adsorbed to immunoplate wells and number of the cells was estimated with the fluorescent peptide, the fluorescence of each well was proportional to the added cell number in a range of \(10^5 - 10^8\) cells.

The peptide is advantageous over antibody in the fluorescence polarization assay (FPA) since sensitivity of the FPA is maximized when a fluorophore is linked to a small molecule. In the FPA using the fluorescent peptide, the FP value increased with increasing amount of *E. tarda* in a range of \(5.2 \times 10^3 - 2.1 \times 10^5\) cells whereas no significant change was observed with *Escherichia coli* and *Yersinia enterocolitica*.

We devised a two step process to detect *E. tarda* with the peptide containing a biotin group and a quartz crystal microbalance (QCM) biosensor connected to a filter-elution module. At first, a mixture of *E. tarda* and the biotinylated peptide was injected into the module where the *E. tarda*-
peptide complex was separated from unbound peptides by filtration. Secondly, the complex was detected with a streptavidin-coated QCM sensor chip. The samples containing the biotinylated peptide and \textit{E. tarda} showed concentration dependent frequency change in a range of $8 \times 10^2 - 8 \times 10^6$ cells while control samples caused no significant frequency change.

These results show the peptide can be used for sensitive detection of \textit{E. tarda} by fluorometric and biosensor methods.

Keywords: Edwardsiella tarda, phage-peptide library, fluorescence polarization, quartz crystal microbalance