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Capture and detection of *Staphylococcus aureus* with dual labeled aptamers to cell surface components

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ABSTRACT

In the present study, a high throughput whole cell SELEX method has been applied successfully in selecting specific aptamers against whole cells of *Staphylococcus aureus*, a potent food poisoning bacterium. A total ten rounds of SELEX and three rounds of intermittent counter SELEX, was performed to obtain specific aptamers. Obtained oligonucleotide pool were cloned, sequenced and then grouped into different families based on their primary sequence homology and secondary structure similarity. FITC labeled sequences from different families were selected for further characterization via flow cytometry analysis. The dissociation constant (K_d) values of specific and higher binders ranged from 34 to 128 nM. Binding assays to assess the selectivity of aptamer RAB10, RAB 20, RAB 28 and RAB 35 demonstrated high affinity against *S. aureus* and low binding affinity for other bacteria. To demonstrate the potential use of the aptamer a sensitive dual labeled sandwich detection system was developed using aptamer RAB10 and RAB 35 with a detection limit of 10^2 CFU/mL. Furthermore detection from mixed cell population and spiked sample emphasized the robustness as well as applicability of the developed method. Altogether, the established assay could be a reliable detection tool for the routine investigation of *Staphylococcus aureus* in samples from food and clinical sources.

1. Introduction

S. aureus is an important pathogen due to combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (Normanno et al., 2005; Argudín et al., 2010). Besides being the important causative agent of food poisoning by the virtue of staphylococcal enterotoxins (SEs), it also causes numerous diseases ranging from minor skin infection to life threatening septicemia, endocarditis (Robertson et al., 1958; Murray, 2005; Kapral et al., 1980). Very small doses of SEs approximately 0.5 ng/mL can cause outbreaks and are considered as superantigens (Roussel et al., 2015; Cross et al., 1983). *S. aureus* has acclimatized rapidly to the selective pressure of antibiotics and emerged as methicillin-resistant *S. aureus* (MRSA), vancomycin resistant *S. aureus* (VRSA) (Klein et al., 2007; Ortega et al., 2010). Hence management of *S. aureus* outbreaks has gained socio-economic significance globally underlining the need for a rapid method of detection.

Gold standard for the detection of *S. aureus* is based upon lengthy, complicated conventional culturing followed by biochemical characterization. Virulence potential of the strain can be done by PCR and quantitative RT-PCRs (Blaiotta et al., 2004). SEs and other toxins can be detected by immunological methods such as enzyme immunoassay and enzyme-linked fluorescent assays (Huang and Chang, 2004; Timofeyeva et al., 2014). However, these methods have variation in their selectivity and require prior isolation of bacterial DNA, protein/toxin and sophisticated instruments. Moreover, preparation of a specific antibody is a time-consuming, expensive, complicated process, and lack of stability in some environment limits their applications.

To overcome these, aptamers are emerging as alternative key with great demand in the various fields like medical field as a therapeutic agent; pharmaceutics for drug discovery and validation and in the field of diagnostics as a biological recognition elements (Jayasena, 1999; Strehlitz et al., 2012). Aptamers are single stranded DNA or RNA which bind to target molecules by changing its conformation with affinity (Radom et al., 2013). Aptamers are becoming more attractive as it offers various advantages in terms of stability broad range of temperatures, pH, chemicals and solvents; lower molecular weight, higher multiplexing capabilities, ease of synthesis, consistent lot to lot performance and lower cost of production in comparison to antibodies (M. Meyer et al., 2013; Setlem et al., 2016; Mondal et al., 2015; S. Meyer et al., 2013; Cibiel et al., 2011).

Aptamers specific to a wide range of protein and non-protein targets have been identified by systematic evolution of ligands by exponential enrichment method (SELEX) (Setlem et al., 2016; Mondal et al., 2015). Some studies have suggested that using live pathogen whole-cells can

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0168-1605/

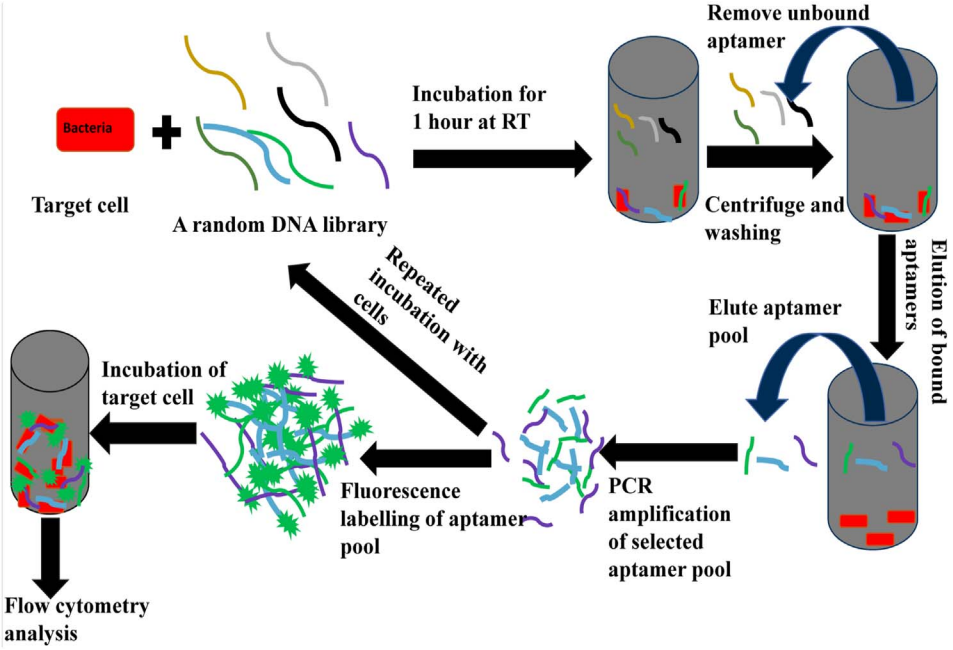

Fig. 1. Illustration of the whole cell SELEX method for selection of aptamer against *S. aureus*.

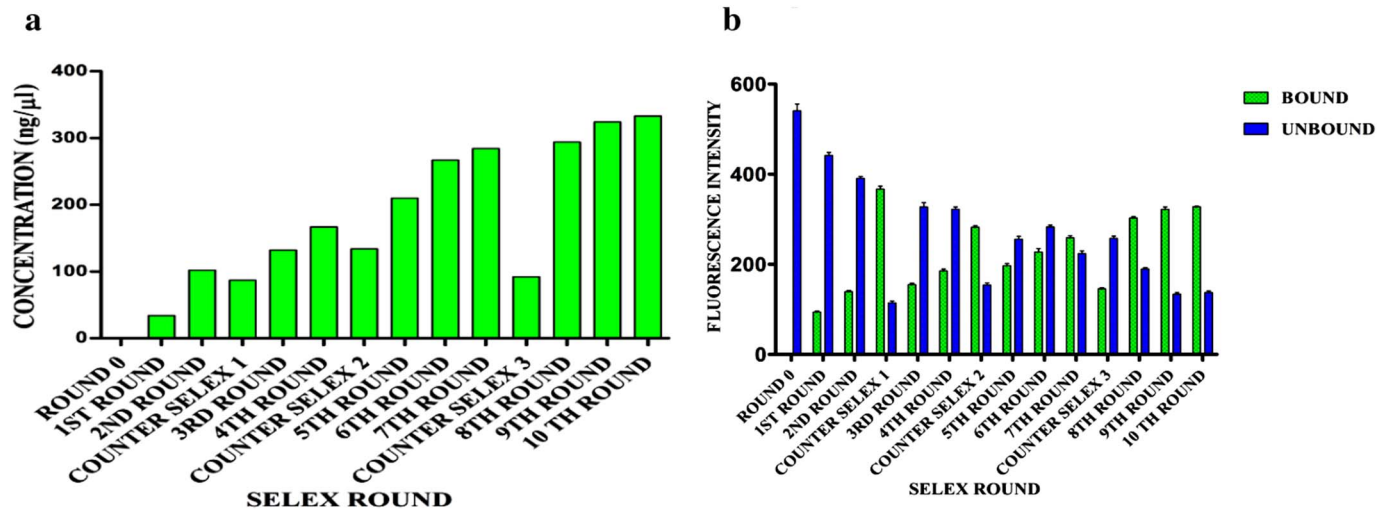

Fig. 2. Bound DNA concentration and Fluorescence intensity of bound and unbound aptamers during the selection process. Round 0 represents the naive library.

be targeted in SELEX, in which case the entirety of the cell surface is presented as a target in native conformation can actually be faster, easier, and more reproducible, with greater success in generating functional aptamers with high binding affinity to live cells (S. Meyer et al., 2013; Cibiel et al., 2011). Screening of aptamers against different bacteria is gaining attention from past two decades (Cao et al., 2009; Baumstummler et al., 2014; Dwivedi et al., 2010; Suh et al., 2014; Lavu et al., 2016; Dwivedi et al., 2013; Moon et al., 2013; Suh and Jaykus, 2013; Chang et al., 2013; Moon et al., 2015).

In this study, we selected aptamers against live whole cells of *S. aureus* by utilizing whole-cell SELEX method, closely related and other bacterial cells as targets for negative selection. Binding constants, selectivity and specificity of individual aptamers were assessed by flow cytometry analysis. A sandwich fluorescence assay was developed to demonstrate the potential use of the aptamers for capturing bacteria and its direct detection. The specificity and sensitivity studies were carried out employing *S. aureus* and other related bacterial strains. The robustness of the developed assay was assessed using mixed bacterial population and artificially spiked samples.

The molecules bound by each aptamer were not identified, but are likely to be found among the lipopolysaccharide, outer membrane proteins, or surface-displayed lipoproteins.

2. Material and reagents

The initial ssDNA library, aptamer sequences and the primers used in this study were obtained from Xcelris Bioscience (Ahmadabad), except where mentioned specifically (Supporting Table 1). All the solutions were prepared with ultra-high purity water. The chemicals and solvents used in present study are listed in the Supporting information 1.

3. Bacterial cultures and harvest conditions

The bacterial strains used in the study were four strains of *S. aureus* (RAB 9001, ATCC 700699, 6538P, 25923), *B. subtilis* (ATCC 21336), *C. freundii* (ATCC 8090), *E. coli* (ATCC 43896), *K. pneumonia* (ATCC 13883), *L. monocytogenes* (ATCC 19112), *P. aeruginosa* (ATCC 27853), *S. flexneri* (ATCC 29903). *Staphylococcus aureus* were cultured with Brain-Heart infusion broth (Himedia, India) at 37 °C; *B. subtilis* was