Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2021, 13(6):01-05

Mini-Review

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Screening of Bacterial Display System and its Applications

Narendra Kumar S, Shivaprada S Hegde*

Department of Biotechnology, R V College of Engineering, Bangalore, India

---

ABSTRACT

Bacterial surface display system was developed to surface expose heterologous protein or peptide for various biotechnological and industrial application. Various outer membrane proteins such as FadL, LamB, LppOmpA, OmpA, OmpC, OmpF, OmpS, OprF and PhoE, have been used to display various peptides and proteins, including small molecular weight peptides, antibodies, domains, enzymes and receptors. The majority of the previously developed systems are only suitable for peptides or relatively small polypeptides. Therefore, the success of a bacterial cell surface display system is highly dependent on the choice of an anchoring motif that is appropriate for the intended target protein. Bacterial cell surface display is advantageous because the use of bacterial cells simplifies the polypeptide selection method and enables fast screening of potential recognition elements typically using fluorescence activated cell-sorting (FACS) and/or Magnetic Activated Cell-Sorting (MACS). This mini review mainly focus on construction of bacterial display, various scaffolds used for library screening and applications of bacterial display.

Keywords: Bacterial display system; Protein engineering; Polyclonal antibodies

---

INTRODUCTION

Nanotechnology Bacterial display system is a protein engineering technique used for display of target such as peptide or protein (enzyme) on the surface of the bacteria. Display of heterologous protein or peptide on bacterial surface was first reported in 1986 [1]. Bacterial display system have been employed for various biotechnological and industrial application such as biocatalysis, peptide library screening, live bacterial vaccine production, biosorbents and biosensor development. In particular, *Escherichia coli* display systems have been widely used because large libraries can be constructed efficiently in *Escherichia coli*, which is ideal for display libraries because it grows quickly and is easy to manipulate [2].

A variety of display systems have been developed in past 30 years. A typical cell source display system includes a carrier protein that act as an anchoring motif and a passenger protein or peptide that act as a target protein. In particular, *Escherichia coli* display systems have been widely used because large libraries can be constructed efficiently in *Escherichia coli*, which is ideal for display libraries because it grows quickly and is easy to manipulate [3]. However, cell surface display system encounters difficulty in displaying a large protein stably and with the correct steric conformation because the protein must pass through two membrane layers and then undergo protein targeting. Thus, the choice and design of anchoring motifs are highly important for the stability of cell envelope integrity. Various anchoring motifs such as outer membrane proteins (OMP), lipoproteins, autotransporters, subunits

of surface appendages and S-layer proteins have been examined [4]. Among the different anchoring motif options, outer membrane proteins, such as FadL, LamB, LppOmpA, OmpA, OmpC, OmpF, OmpS, OprF and PhoE, have been used to display various peptides and proteins, including small molecular weight peptides, antibodies, domains, enzymes and receptors [5]. Each anchoring motifs has been found to have different capacities for protein display making it necessary to develop appropriate anchoring motifs for proteins of various sizes and characteristics.

The development of a bacterial display system suitable for robust reagent discovery has proven challenging. There are three main challenges in creating a high speed affinity ligand isolation technology against unknown/un-catalogued pathogens: (1) creation of a large (high diversity) and robust cell based library (2) creation of an ultrahigh throughput, disposable screening system, and (3) gaining a fundamental understanding of the factors which influence binder performance along with this understanding the development of methodologies to enable universal isolation and optimization of ligand binder performance [6].

PROTEIN ENGINEERING WITH BACTERIAL DISPLAY

The most commonly used host for bacterial display is *Escherichia coli* because of its various advantages such as rapid growth rate, ease of genetic and physical manipulation, and its suitability for making large libraries of up to 1011 members. Although a wide variety of different scaffolds have been used to display protein or peptide on the surface of *E. coli*, only few scaffolds have been used for library screening (Table 1).

Table 1: Bacterial display scaffolds used for library screening

Display scaffold	Display library	Targets
OmpA	Insertional fusion; semirandom 9 a.a. peptide, random 12 a.a./15 a.a. peptide	HIV GP120, CRP, T7 mAb, HSA, streptavidin, tumor cell,red blood cells
OmpX	Insertional fusion; random 15 a.a. peptide	Monoclonal antibodies
Invasin	C-terminal fusion; random 10 a.a. peptide	Human cell lines
Lpp	C-terminal fusion; antidigoxin scFv antibody libraries	Digoxin-fluorescent conjugate
Autotransporters: AIDA-I, IgA protease, CPX	N-terminal fusion; random 15 a.a. peptide, semirandom 28 residue cystine knot peptide N-terminal fusion; 7 a.a. constrained peptide, random 15 a.a. peptide	Cathepsin G, trypsin Streptavidin, Tumor cells

The surface display of passenger proteins on *E. coli* are often achieved by genetic fusion with various 'scaffold' proteins targeted to the outer membrane and also to those assembled into flagella and fimbrial structures. Bacterial display scaffolds are broadly grouped into those allowing N-terminal, C-terminal, and insertional fusions. Display of passenger polypeptides as N-terminal fusions with a surface-exposed N-terminus of the display scaffold can be achieved *via* fusion to autotransporter proteins. Display *via* the scaffold's C-terminus is beneficial to enhance the diversity of peptide libraries since stop codons arising from common randomization schemes and non-intended errors (primer deletions or PCR errors) can yield functional binders without truncating the carrier protein. C-terminal display libraries have been generated and screened using intimins (EaeA), invasins, and the LppOmpA vector [7].

A scaffold presenting both N-terminal and C-terminal on the cell surface was recently engineered by circular permutation of the smallest member of the outer membrane protein family, OmpX. The circularly permuted OmpX (CPX) scaffold enables normalization of protein display levels by fluorescence labeling of a C-terminal affinity tag.

Among all these approaches, display of peptides on bacterial flagella using 'FliTrx' system have been used most because of its commercial availability. In FliTrx system peptide are presented within the active site loop of *E. coli* thioredoxin which is in tern inserted into a repeated flagellar protein FliC. Insertion libraries such as FliTrx and those created in outer membrane proteins (e.g. OmpA, OmpC, OmpX, and FhuA) are well suited for mapping antibody and protein-binding epitopes, and selecting initial low affinity binders toward challenging targets for subsequent affinity maturation [8].

SCREENING OF BINDING LIGANDS

Screening of cell surface display libraries generally require Fluorescent Activated Cell Sorting (FACS). Since use of magnetic selection (MACS) alone or panning processes such as that used with the FLiTrx system lead to avidity interactions that interfere with affinity screening (Figure 1). When screening on the basis of dissociation kinetics using FACS the use of a large excess of a nonfluorescent competitor (i.e. nonbiotinylated target protein) may be necessary to prevent rebinding effects that can enhance apparent affinities measured for cell displayed ligands.

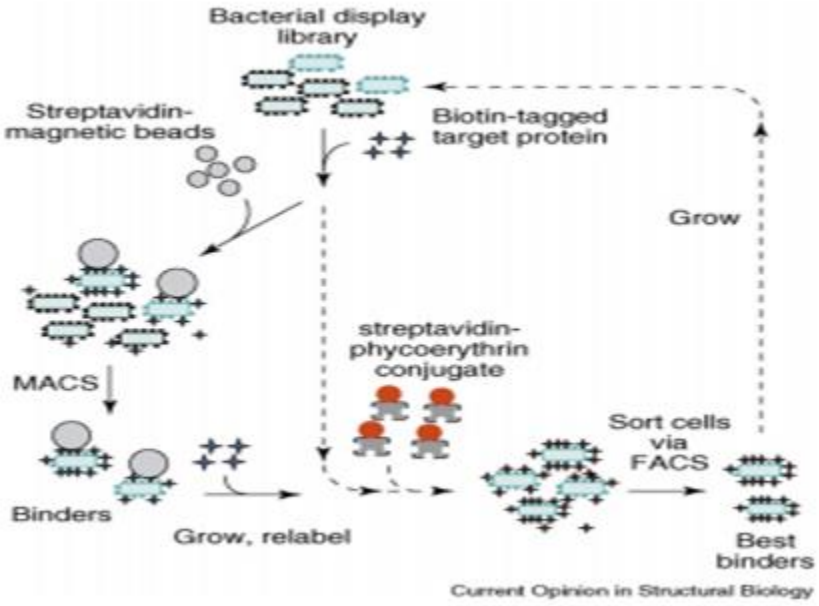

Figure 1: Bacterial display library screening

Typical combined selection and screening strategy for large (10^8 to 10^{11}) libraries using biotinylated target proteins for sequential magnetic separation (MACS) with streptavidin-functionalized magnetic particles followed by fluorescence-activated cell sorting (FACS) of the enriched population for fine affinity resolution.

APPLICATIONS

Epitope Mapping of both Monoclonal and Polyclonal Antibodies

This is achieved by determination of antibody-binding epitopes by using an antigen-focused, library-based approach in conjunction with flow cytometric sorting (Figure 2).

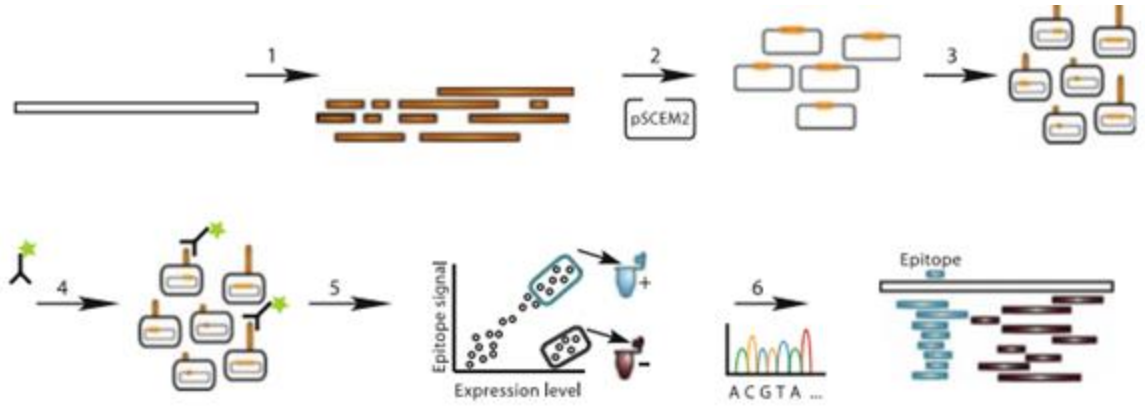

Figure 2: Epitope mapping of both monoclonal and polyclonal antibodies

The gene encoding the target antigen to be mapped is amplified by PCR and fragmented (1) followed by cloning into the staphylococcal display vector (pSCEM2) (2). The library containing plasmids are transformed into *Staphylococcus carnosus*, where in frame fragments are displayed on the bacterial surface (3). After incubation with antibody (4), binding and non-binding cells are sorted using FACS to isolate epitope and non-epitope containing cells (5). Colonies derived from individual cells are sequenced (6), and sequences from epitope (blue) and non-epitope (red) binding cells are aligned back to the antigen in order to together determine the antibody epitope.

The library members are generated by fragmentation of the target antigen DNA, and the Gram-negative bacterium *E. coli* is first used for preparation of the library containing plasmids. The library is then presented as peptides on the cell surface of the non-pathogenic Gram-positive bacterium *Staphylococcus carnosus*. The cell surface anchoring domain is located downstream of the epitope peptides in the expression cassette; hence, only in-frame fragments are analyzed since out-of-frame regions cannot bind to the membrane nor be detected by labelled HSA. Epitope expression level is monitored by measuring labelled HSA binding to the albumin-binding domain (ABD) downstream of the epitope sequence, on the cell surface just before the membrane-anchoring region. This organism allows for high multivalent surface expression, rapid library screening and sorting of antibody-binding cells using flow cytometric devices thanks to its rigid cell structure. In contrast to conventional epitope mapping methods, this method offers a powerful and efficient way to map continuous epitopes by screening both long and shorter antigen fragments in one library suitable for both monoclonal and polyclonal antibodies.

Displaying Different Enzymes of Interest for Biotechnological and Industrial Application

One of the studies illustrated that, *E. coli* surface display system achieved by using anchoring motif derived from protein YiaT. YiaT is putative outer membrane protein and its function has been not characterised previously. Therefore, to determine best surface anchoring protein full length YiaT and two of its C terminal truncated forms, cut at R181 and r232 has been evaluated. Two industrial enzymes, a lipase from *Pseudomonas fluorescens* SIK W1 and an α-amylase from Bacillus subtilis, were used as the target proteins for display. *E. coli* XL-1 Blue was used as the host strain for general cloning, and the three *E. coli* strains XL-1 Blue, XL10-Gold and W3110 were used as the host strains for the cell surface display studies [9].

In this study, the C-terminal deletion–fusion strategy was used to display the enzymes. The full-length yiaT gene and the C-terminal truncated yiaT (yiaTt) genes encoding the first 181 and 232 amino acids (from the N-terminus) were amplified by PCR. The genes were cloned into the EcoRI and XbaI sites of pTrc99A. To display lipase gene on *E. coli* surface the *Pseudomonas* lipase gene containing the FLAG (DYKDDDDK) was amplified. It was then

cloned into the XbaI and HindIII sites of the plasmid. To display amylase α-gene on *E. coli* surface, the B. subtilis α-amylase gene containing the FLAG sequence was amplified it was then cloned into the XbaI and HindIII sites of the plasmid. Display of lipase on recombinant *E. coli* was verified by enzymatic activity and SDS-PAGE. Display of α-amylase on recombinant *E. coli* was verified by western blotting. Results showed that highly active forms of lipase and α-amylase without any cell growth defects were able to successfully display on *E. coli* surface.

This system containing truncated YiaT anchor proteins was able to overcome the significant problems associated with the broader applications of display systems, such as target protein size limitations and misfolding. Therefore, this system can be used to display different enzymes of interest, making it possible to develop cost-effective biocatalytic systems for a variety of chemical industries [10].

Oral Vaccine Development

Oral vaccines are an attractive medical application of cell surface engineering technology. After the discovery of the effectiveness of vaccines, several fundamental methods to produce antigenic proteins have been developed. Recently display and evaluation methods for production of oral vaccines using cell surface engineering have been established. *Saccharomyces cerevisiae* and *Lactobacillus casei* have been used to display antigens and studied as delivery tools for antigens. These microorganisms function not only as vehicles for antigens but also as adjuvant materials.

CONCLUSION

Bacterial display methodologies are poised to make important contributions in peptide and polypeptide engineering. Combining the simplicity of bacterial cell manipulation, with large libraries, and quantitative screening *via* FACS, these approaches have potential to streamline the identification and optimization of binding ligands. Bacterial display in conjunction with multi parameter FACS should be particularly useful for the screening and optimization of a variety of emerging non-antibody therapeutics derived from small scaffolds A key advantage of bacterial display technology over traditional antibody creation as well as other peptide display alternatives is that it offers an strategy for generating tailor-made affinity ligands in a very short time period of several days.

REFERENCES

[1] Daugherty PS. *Curr Opin Struct Biol*. **2017**; 17(4), 474-80.

[2] Bessette PH. Rice JJ, Daugherty PS. *Protein Eng Des Sel*. **2004**; 17, 731-739.

[3] Volk AS, Jingxin Hu, Rockberg J. *Spriger science*. **2014**; 1131,485-500.

[4] Chi-Lung AL, Harris JL, Khanna K, Ji-Hong H. *Int J Mol Sci*. **2019**; 20(10),2383.

[5] Getz JA, Schoep TD, Patrick S. *Methods Enzymol*. **2017**; 503,75-97.

[6] Shivange AV, Patrick S. *Methods Mol Biol*. **2015**; 1248,139-53.

[7] Henninot A, Collins JC, John M. *J Med Chem*. **2018**; 61(4), 1382-1414.

[8] Mattanovich D, Borth N. *Microb Cell Fact*. **2006**; 5,12.

[9] Bessette PH, Rice JJ, Daugherty PS. *Protein Eng Des Sel*. **2004**; 17, 731-739.

[10] Shibasaki S. *Methods Mol Biol*. **2016**; 1404, 497-509.