

Review Article

Microbial cell surface display; its medical and environmental applications

Vida Tafakori^{#1}, Ibrahim Torktaz^{#2}, Mohsen Doostmohammadi², Gholamreza Ahmadian^{*1}

¹Department of Molecular Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), P.O. Box 14965/161, Tehran, I.R. Iran ²Department of Biotechnology, Faculty of Advanced Science and Technologies, University of Isfahan, 81746-73441, Isfahan, I.R. Iran

Received: 28 January 2012

Accepted: 29 April 2012

Abstract

Cell-surface display is the expression of peptides and proteins on the surface of living cells by fusing them to functional components of cells which are exposed to the environment of cells. This strategy can be carried out using different surface proteins of cells as anchoring motifs and different proteins from different sources as a passenger protein. It is a promising strategy for developing novel whole cell factories. Surface engineered cells have many potential uses ranging from medical to environmental applications. This review focuses on different strategy and applications of microbial surface display.

Keywords: Environmental; Medical; Microbial Surface Display

INTRODUCTION

Genetic engineering was established in the 1970s. since then different methods for engineering microorganisms with special abilities have been developed. One of these methods is a technology called molecular display (Arming) technology or cell surface engineer-

ing. This technology allows displaying proteins or peptides on living cells such as yeast, mammalian and bacterial cells (Shibasaki *et al.*, 2009). This technology enables us to produce modified cells with desired surface properties. The first technology, phage display, was set up in the 1980s (Smith, 1985). Bacterial cell surface display was then established in the 1986s (Charbit *et al.*, 1986). Because of its advantages such as easy proliferation, large cell size and independent life style of bacteria, bacterial cell surface display progressed rapidly. However, the expression of heterologous eukaryotic proteins was sometimes difficult by this system. some of these proteins need post-translational modifications such as glycosylation, phosphorylation and so on. In these cases, yeast cells are good candidates.

Surface display of heterologous proteins on the cell surface of microorganisms is useful for developing different whole-cell factories with different applications including separation of produced polypeptides; production of whole cell biocatalysts, whole-cell bioadsorbents, live vaccines and screening of modified or novel proteins. Utilization of the surface of living cells is attractive in different microbiology and molecular biology applications (Ueda and Tanaka, 2000 a, b).

In this review, we focus on the principles and some applications of molecular display technology in yeast

*Correspondence to: Gholamreza Ahmadian, Ph.D.

#Authors equally contributed to this paper

Tel: +98 21 44580351; Fax: +98 21 44580395

E-mail: ahmadian@nigeb.ac.ir

and bacteria in medical and environmental fields.

Principle of cell surface engineering in bacteria and yeast

The cell surface is a functional part of the cell and can mediate many important cell functions. Cell surface proteins may extend across the plasma membrane and other may non-covalently or covalently interact with cell-surface components. In biotechnology, cell surface components can be used as carrier proteins to display heterologous proteins (passenger proteins) on the cell surface. The passenger protein can be fused to carrier protein C-terminally or N-terminally. The N-terminal fusion is used when the carrier protein has an anchoring domain in its C-terminus part. The C-terminal fusion is used when anchoring domain is in N-terminal of carrier proteins. So various gene fusion strategies have been considered in bacteria: C-terminal fusion, N-terminal fusion and sandwich fusion (Fig. 1).

In Gram-negative bacteria, cell membrane consists of cytoplasmic membrane, periplasmic space and outer membrane. Anchoring proteins in Gram-negative bacteria should pass through these layers to the surface of the cell. The Lpp'–OmpA hybrid is a good example of C-terminal fusion type. This system was first devel-

oped by Georgiou *et al.*, (1996). The anchoring part of this system include the signal sequence and nine N-terminal residues of the mature *E. coli* lipoprotein, Lpp', fused to the residues 46-159 of the *E. coli* outer membrane protein A (OmpA). The passenger proteins will then be cloned, expressed and exposed to the environmental side of the cell as a C-terminal fusion to this anchor (Georgiou *et al.*, 1996).

Tafakori *et al.* (2012) used a modification of this system by fusing Lpp'–OmpA chimera to cyanobacterial metallothionein and chitin binding domain of chitinas S from *Bacillus pumilus* SG2 (Ahmadian *et al.*, 2007) for metal adsorption and cell immobilization. They showed that this system function efficiently as a bioadsorbent. Peptidoglycan-associated lipoprotein (PAL) is an example of N-terminal carrier protein. PAL binds to the peptidoglycan layer and outer membrane with its C-terminal portion and N-terminal, respectively (Dhillon *et al.*, 1999).

The most commonly strategy used for the surface display of proteins in Gram-negative bacteria is sandwich fusion. Outer membrane proteins (OMPs) are common carriers in this system. These β -barrel structures consist of antiparallel β -strand pairs and two types of loops: external less conserved loops and periplasmic conserved loops. The external loops can be used as a carrier for sandwich strategy (Lee *et al.*,

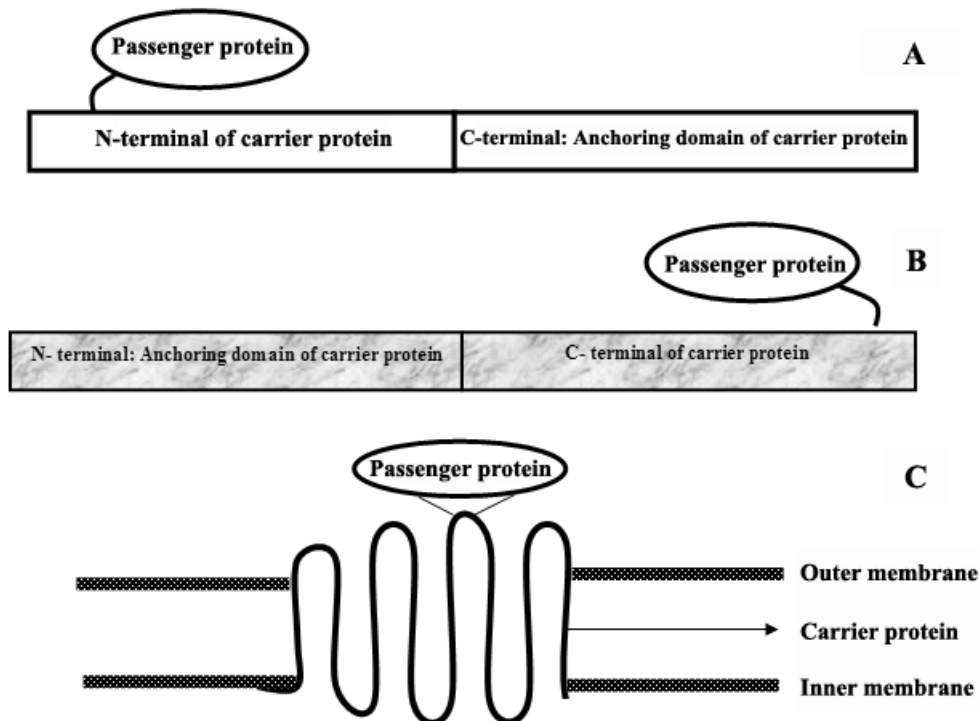


Figure 1. Various gene fusion strategies in bacteria. A: N-terminal fusion. B: C-terminal fusion. C: Sandwich fusion.

2003). OmpC is a putative example of this strategy. Xu and Lee, (1999) successfully inserted a poly-histidine peptides containing 162 amino acids, into the seventh external loop of OmpC.

In Gram-positive bacteria, cell envelope composed of one biological membrane and a cell wall structure containing peptidoglycan and covalently linked teichoic acids. Different surface proteins, which are part of the cell envelope, have been used for surface display of proteins in gram positive bacteria. The main types of these are: (i) transmembrane proteins, (ii) lipoproteins, (iii) LPXTG-like proteins, (iv) cell wall-binding proteins and (iv) Macromolecular protein surface structures (Desvaux *et al.*, 2006). These carrier proteins are fused to passenger proteins by N-terminal fusion or C-terminal fusion.

In general transmembrane spanning domain of any membrane protein may be used as an anchoring domain in surface display of proteins. The target protein is then linked at its N-terminus to one or more transmembrane spanning domain of a cytoplasmic membrane protein.

Lipoproteins are attached to the peptidoglycan and serve important function in adhesins, transporters, receptors, enzymes or virulence factors (Sutcliffe and Russell, 1995). Stover *et al.* (1993) fused the outer surface protein A (OspA) of *Borrelia burgdorferi* to the relevant N-terminal part of the *Mycobacterium tuberculosis* lipoprotein Mtb19 in *Mycobacterium bovis* strain bacille Calmette-Guerin (BCG). The OspA surface exposed and the recombinant BCG strain was evaluated in vaccination studies.

Sortase is a transpeptidase that conjugate some surface proteins with LPXTG motif at their c-terminal on

the external side of cell wall of some gram positive bacteria. Fasehee *et al.* (2011) were able to display chitinase (chiS) of *B. pumilus* SG2 chitinase on the surface of *B. subtilis* using a putative sortase, yhcS. They also showed for the first time that this sortase of *B. subtilis* is functional (Fasehee *et al.*, 2011; Ahmadian *et al.*, 2007). The displayed chitinase by the above methods was functional as shown by enzyme assay.

Tsuchiya *et al.* (1999) by fusing cell wall binding (CWB) domain of the major *B. subtilis* autolysin to *B. subtilis* extracellular lipase B, showed that the enzyme binds efficiently but in small amount, to the *B. subtilis* cell wall. They mentioned that, the low level of surface expression is due to the presence of extracellular proteases. To solve this problem and increase the amount of surface displayed lipase, they used strains of *B. subtilis* deficient in some cell-wall binding and extracellular proteases (Kobayashi *et al.*, 2000).

Extracellular structures such as pilus, S-Layer, cellulosome and flagellum consist of subunits that can be used as a carrier protein. For example pilus has occasionally been used as an anchoring motif to express foreign peptides on the surface of bacteria by inserting the peptides into its permissive sites. Saffar *et al.* (2005) developed a bioadsorbent by expressing a short cysteine rich peptide on the surface of enterotoxigenic *E. coli*. They used CS3 fimbriae as a carrier and showed that the ability of the bacteria to adsorb Ni²⁺ and Cd²⁺ ions was increased five-fold and three-fold compared to the wild type *E. coli*, respectively.

Another living system that can be used for protein surface display, is the bacterial spore and because spores are resistant to environmental stresses, they are a unique opportunity for surface display (Medaglini *et*

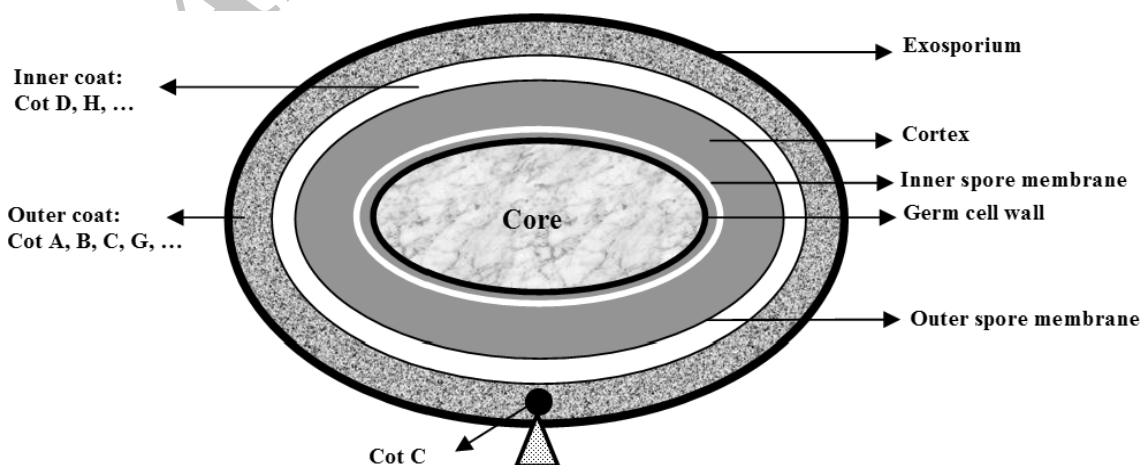


Figure 2. Spore layers and surface display of β -galactosidase on *B. subtilis* spore. Cot C used as an anchoring protein.

al., 2001). In the case of some spore-forming bacteria, ex; *B. subtilis*, both forms including vegetative and spore forms of the bacterial cell, were used for developing various surface display systems. Tavassoli *et al.*, (2012) used spore surface display (CotC) for expression of β -galactosidase (*lacA*) gene from *B. subtilis* strain 168 on the surface of *B. subtilis* strain RH101 (Δ cotC) spores (Fig. 2). The expression of the *lacA* on the spore surface was shown by western blotting, immunofluorescence. β -galactosidase assay was also performed to show that fusion enzyme exposed on the spore surface is functional. They concluded that the immobilized enzyme can be efficiently used as whole cell biocatalyst.

Kwon *et al.* (2007) expressed β -galactosidase on *B. subtilis* spores by fusion it to the spore CotG protein to make a whole-cell biocatalyst. They suggested that this system is very efficient in water-solvent biphasic reaction systems and the hydrophobicity of the spore surface should have facilitated localization at the interface between the two phases. Therefore, his system not only stabilizes the enzyme but also increases availability of β -Gal at the interface.

When it was first introduced in 1997, the engineered yeast cell displaying proteins on its surface was named “arming yeast” (Shibasaki *et al.*, 2009). Agglutinins and Flo1 are the main components that are

generally used for displaying proteins on the yeasts cell surface. Agglutinin proteins, a-agglutinin and α -agglutinin, are expressed on the surface of *S. cerevisiae* and involved in mating of the a- and α -cells, respectively. The *AGa 1* gene encodes α -agglutinin and this protein interacts with the binding subunit of a-agglutinin of a-type cells. AGA1 and AGA2 are core and binding subunits of a-agglutinin, respectively. These subunits are linked together through disulfide bridges (Zou *et al.*, 2000). α -agglutinin and the core subunit of a-agglutinin consist of four parts: A secretion signal, an active region, a serine-threonine rich support region and GPI anchor attachment signal (Kondo and Ueda, 2004). For surface display of proteins, they are combined with the GPI-anchor attachment signal at C-terminal end of α -agglutinin (Fig. 3A). For anchoring protein using a-agglutinin system, the binding subunit of the protein Aga2p binds to heterologous protein by its C-terminus and to Aga1p by its N-terminus (Fig. 3B). As the Aga2p fusion protein and Aga1p associate within the secretory pathway, this construct exports to cell surface and links to the cell wall.

Flo1 protein encoded by *flo1* gene and is a serine-threonine-rich- lectin-like cell- wall protein of *S. cerevisiae*. This protein is composed of four main domains including flocculation domain, secretion signal, glycosyl phosphatidylinositol (GPI) anchor attachment sig-

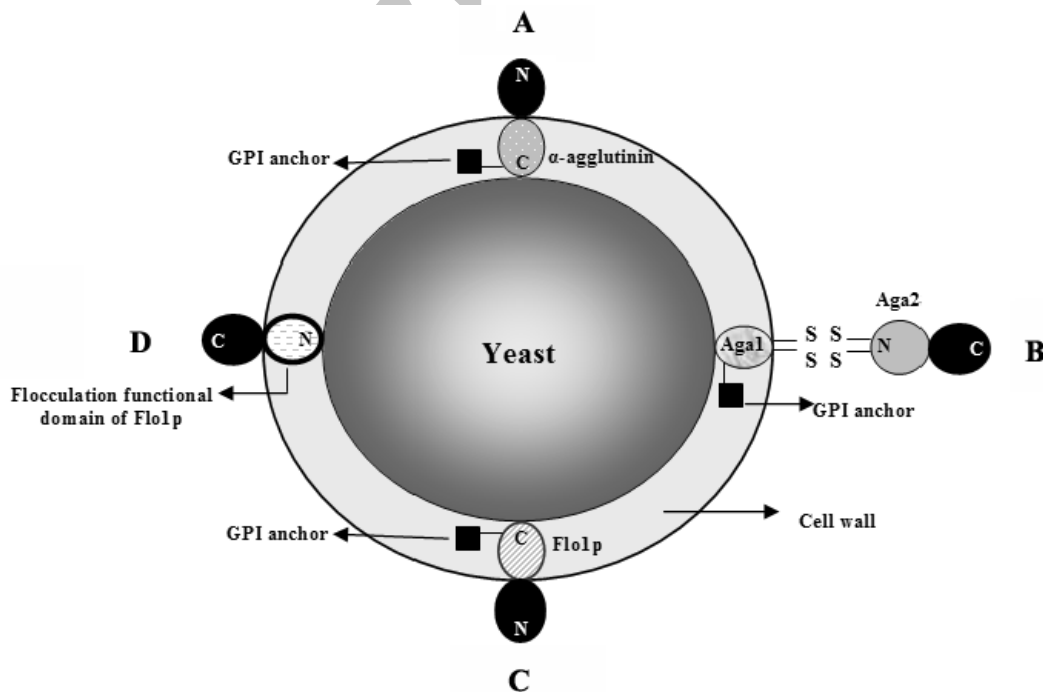


Figure 3. Yeast surface display systems. A: α -agglutinin system, B: a- agglutinin system, C: Flo1p system and D: flocculation domain of Flo1p. Black ovals are passenger proteins.

nal, and membrane-anchoring domain (Kondo and Ueda, 2004). Truncation of Flo1 polypeptide indicated that the hydrophobic C-terminal domain of this protein, contains a GPI-attachment signal, is necessary for anchoring of Flo1p in the yeast cell wall (Bony *et al.*, 1997). GPI-anchoring domain of Flo1p is used as a common surface display system in yeast (Fig. 3C). Meanwhile, the adhesive ability of the flocculation domain of Flo1p is used for surface display of proteins in yeast display system (Matsumoto *et al.*, 2002) (Fig. 3 D).

Environmental application of surface display

There are two kinds of pollutants: organic and inorganic materials. Organic pollutants contain insecticide, pesticides, volatile organic compounds, oils, poly aromatics hydrocarbons, dyes, etc. Inorganic pollutants include fertilizers and metals. These can cause serious damages to human health and other organisms. Organic contaminations can be degraded by enzymatic activity and inorganic contaminations can be removed by uptake or adsorption strategy.

Enzymatic degradation of organic contaminants can be performed by the cells containing degradative enzymes, either free or immobilized on their surface. The advantages of immobilized over soluble enzymes arise from their enhanced stability and ease of separation from the reaction media, leading to significant savings in enzyme consumption (Tischer and Kasche, 1999). Different conventional methods used to immobilize enzymes, involve covalent, non covalent or inclusion interaction of free enzymes together or with a carrier (Tischer and Kasche, 1999). However all of them need enzymes purification step which leads to cost increasing. Expression of recombinant enzymes on the surface of live bacteria, bacterial spore and yeast, solve this problem. This is used to make a whole cell and recyclable biocatalyst (Rutherford and Mourez, 2006). So far many diverse enzymes are displayed on the bacterial cell surface. These include β -galactosidase (Xu *et al.*, 2011; Kwon *et al.*, 2007) chitinase (Wu *et al.*, 2006), amylase (Narita *et al.*, 2006), lipase (Jung *et al.*, 2006), etc. A thermostable α -amylase from *Bacillus licheniformis* was immobilized on the surface of *Bacillus subtilis* spore as carrier using covalent attachment by non-genetically method. Catalytic properties and stability of the immobilized α -amylase were improved when compared with that of free enzyme. The optimum pH and temperature was determined and shown to be different for free and

immobilized enzyme (Gashtasbi *et al.*, 2012). The results showed potential of immobilized amylase, for starch degradation.

Different researchers displayed diverse enzymes on *S. cerevisiae* cells. Murai *et al.* (1997) anchored *Rhizopus oryzae* glucoamylase, on yeast cells by C-terminal half of α -agglutinin and show this yeast cells utilized starch directly as the sole carbon source. Matsumoto *et al.* (2002) used flo1p system, for surface display of lipase from *Rhizopus oryzae* on *S. cerevisiae*. Displayed lipase successfully catalyzed the methanolysis reaction.

Conventional physicochemical methods for removing/recovery of metal ions from contaminated waters such as industrial wastewater are often ineffective and costly when applied to dilute effluents (Kuroda and Ueda, 2006). Microorganisms use two ways to recover metal ions from environment. One way is adsorption of metals to the surface components and another one is gradual accumulation of metals to the cells. Surface adsorption of metals has several advantages: desorption of Metal ions without cell disruption with mild chemical treatment, so reusing of bioadsorbents for the further metal adsorption cycle, elimination of the metal crossing the membrane barrier, thus removing the rate-limiting step and increasing the overall kinetics (Wu *et al.*, 2004), using of dead cells because of independence of metabolism. This facilitates tolerance to higher toxic metal concentrations without damaging to cells (Vijayaraghavan and Yun, 2008).

There have been many researches on microbial surface display of metal binding domains for biosorption of heavy metals. In a study by Tafakori *et al.* (2012) a variation of the previous strategies was used to devise a novel bioadsorbent. A combination of two different cyanobacterial metallothioneins, SmtA or MtnA, and chitin binding domain were used in this research to develop a whole cell bioadsorbent. The authors successfully demonstrated the functionality of the fusion proteins in *E. coli* (Tafakori *et al.*, 2012). Xu *et al.* (2002) previously by expressing a bifunctional fusion proteins consisting of synthetic phytochelatin (EC20) linked to a *Clostridium*-derived cellulose-binding domain (CBD_{cl}), showed that The immobilized sorbents is highly effective in removing cadmium at parts per million levels and enabling immobilization onto different cellulose materials in essentially a single step (Xu *et al.*, 2002). Some researchers have used histidine tag as a model to develop various surface display modules. Hinc *et al.* (2010) engineered *B. subtilis* spores to express eighteen histidine residues as fused to promot-

er and N-terminal part of cotB. By analyzing the wild type and recombinant spores, the researcher showed that the recombinant spore efficiently adsorbed nickel ions, and the recombinant spore proved to be significantly more efficient than wild type spores in metal-binding. The effect of biomass, pH and temperature was evaluated in this study. They suggested that the recombinant spore displaying metal binding domains can be used as a new and potentially powerful tool for the treatment of contaminated ecosystems (Hinc *et al.*, 2010).

To construct bioadsorbents for the removal of toxic metal ions and recovery of rare-metal ions, various metal-binding proteins/ peptides such as hexa-histidine, metallothionein and the transcription factor ModE (with C-terminal molybdate-binding domain) with ability to bind toxic divalent heavy metal ions or rare metals, were successfully displayed on the cell surface of yeast by α -agglutinin-based system. These bioadsorbents show enhanced adsorption of heavy metal ions such as copper, nickel, cadmium and molybdenum ions (Nishitani *et al.*, 2010; Kuroda and Ueda, 2003; Kuroda *et al.*, 2001).

Separation of the bioadsorbents after metal removing/recovery is very important. Cell aggregation results spontaneous separation of cells from the treated water, decreasing in costs and improved simplicity of the procedure. Two kinds of modifications were carried out to increase the usefulness of the cell surface-engineered yeasts in bioadsorption. As the first improvement, the self-aggregation ability in response to environmental copper ions was endowed by introducing the fusion gene consisting of a copper inducible CUP1 promoter and GTS1, the induced expression of which causes cell aggregation (Kuroda *et al.*, 2002). As the second improvement, tandem repeating of yeast metallothionein (YMT) displayed on yeast, to enhance adsorption ability (Kuroda and Ueda, 2006).

The creation of proteins/peptides with specific adsorption ability could hold the promise of the construction of novel bioadsorbents with specific adsorption ability for the recovery of rare metals as well as toxic metal ions in the future.

Medical application of surface display

Surface display has different application in medical area such as vaccine development, epitope mapping, antibody display, and etc. Live attenuated organisms; killed but intact organisms and subcellular components are three most important types of vaccines (Kaufmann,

2001). Delivery of live subunit vaccines has been looked upon as an attractive alternative to the more traditional methods because of ease of production. It is shown that they sometimes elicit long-lasting immunity after only a single immunization (Samuelson *et al.*, 2002). To generate live vaccines, two different types of organisms have been used. One group is normally pathogenic organisms that have been subjected to attenuation. Examples of this group are *Salmonella* spp and *M. bovis* strain BCG. Another group is non-pathogenic commensal or food-grade organisms, such as *S. gordinii*, several staphylococcal, lactic acid bacteria, *B. subtilis* bacteria and its spores, and *S. cervisiae*. Ahmadian and colleagues (2012) used an engineered *E. coli* as a vaccine model against Foot-and-mouth disease. They fused several different Foot and Mouth Disease Virus (FMDV) epitopes containing the immunogenic regions of VP1 to the *E. coli* ompA. The immunogenicity of these recombinant bacteria was tested by immunizing the mice. The results showed extra stimulation in the immune system of the mice with daily feed of these recombinant bacteria.

Searching in the literature showed that different antigens are expressed on the *B. subtilis* spore surface by using different spore coat proteins as a carrier. Isticato *et al.* (2001) expressed the C-terminal fragment of the tetanus toxin (TTFC) on the *B. subtilis* spore surface as a fusion to promoter and part of cotB. They suggested that this system can be used for vaccine delivery. Hinc *et al.* (2010) expressed urease subunit (UreA) of animal pathogen *Helicobacter acinonychis* on the *B. subtilis* spore surface using three different spore coat as carriers. The surface expression was confirmed by different means including western blot, dot blot and immunofluorescence microscopy analysis. This subunit is recognized as a major antigen of *H. Pylori* and induces protection against infection. Therefore, this study aimed at developing an efficient oral vaccine not as a whole cell biocatalyst (Hinc *et al.*, 2010).

There are many studies that used yeasts for antigen presentation. Mischo *et al.* (2003) demonstrated the capacity of yeasts to present tumor-associated antigens in their naturally folded state (Mischo *et al.*, 2003). They expressed colorectal cancer antigen A33 on the yeasts surface, and then for testing the applicability of the yeast system for the detection of specific antibody responses, they expressed the full length NY-ESO-1 protein on the yeast surface. In another study in 2006, Zhu *et al.*, displayed the HL1 gene encoding haemolysin from *Vibrio harveyi* SF-1 in yeast cell sur-

face, and their results demonstrated the haemolytic activity on erythrocytes from flounder (Zhu *et al.*, 2006).

Bowley *et al.* (2007) showed that yeasts are more superior to phages in expression of HIV-1 immune sc-FV cDNA library. However, the major obstacle of yeast surface display for expression of allergens is glycosylation of targets by the high-mannose oligosaccharides, which may bind to IgE (Bowley *et al.*, 2007).

Different organisms displaying random peptide libraries on their surface were utilized for epitope mapping. This method is very easy compared to traditional synthetic methods. Lang and collaborates (2000) cloned various PapG fragments into OmpS in order to characterize the adhesive epitopes responsible for binding to globoside (Lang *et al.*, 2000).

The expression of functional antibodies on the surface of bacteria such as *E. coli* and *staphylococci* has applications such as: (i) creation of whole-cell diagnostic tools for the selection of peptides or recombinant antibody fragments from large libraries; (ii) generation of devices for immunopurification; (iii) whole-cell affinity sorbents; and (iv) targeting cells to specific immunoreactive sites or increase their ability to colonize certain tissues (Samuelson *et al.*, 2002). In general, the characteristics of surface-engineered cell are depended on the type of the displayed protein/peptide. Therefore new potential can be produced by using different kinds of proteins/peptides.

CONCLUSIONS

In this review, we described the principle of microbial surface display technology with the emphasis on yeast, bacteria and some of their applications in environment and medicine. This technology allows displaying of different size of protein molecules, from small to large and from single-subunit proteins to hetero-oligomeric multi-subunits. In addition, several different proteins can be displayed simultaneously. Flo1, Cwps and α -agglutinin are main three ways for yeast surface display. Also different anchoring proteins in Gram-positive and Gram-negative bacteria can be used for displaying heterologous proteins on the surface of bacterial cells.

The range of applications of surface display technology is dependent on the displayed proteins on the surface of microorganisms and covers from medical to environmental applications. The engineered cells are expected to find increasingly wider use as expression

systems are gradually improved and technical hurdles are overcome.

Acknowledgements

Our research that was mentioned in this review was supported by the National Institute of Genetics Engineering and Biotechnology, Iran.

References

- Ahmadian G, Degrassi G, Venturi V, Zeigler D R, Soudi M, zanguinejad P (2007). *Bacillus pumilus* SG2 isolated from saline conditions produces and secretes two chitinases. *J Appl Microbiol.* 103: 1081-1089.
- Ahmadian G, Shamsara M, Karbalayi MA, Hadji khodadad S (2012). Using recombinant *E. coli* as a novel vaccine against foot and mouth disease virus. *22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)*. April 31-May 3, London, U.K.
- Bony M, Thnes-Sempoux D, Barre P, Blondin B (1997). Localization and Cell Surface Anchoring of the *Saccharomyces cerevisiae* Flocculation Protein Flo1p. *J Bacteriol.* 179: 4929-4936.
- Bowley DR, Labrijn AF, Zwick MB, Burton DR (2007). Antigen selection from an HIV-1 immune antibody library displayed on yeast yields many novel antibodies compared to selection from the same library displayed on phage. *Protein Eng Des Sel.* 20: 81-90.
- Charbit A, Boulain J C, Ryterl A, Hofnung M (1986). Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope; expression at the cell surface. *EMBO J.* 5: 3029-3037.
- Desvaux M, Dumas E, Chafsey I, Hebraud M (2006). Protein cell surface display in Gram-positive bacteria: from single protein to macromolecular protein structure. *FEMS Microbiol Lett.* 256: 1-15.
- Dhillon JK, Drew PD, Porter AJR (1999). Bacterial surface display of an anti-pollutant antibody fragment. *Lett Appl Microbiol.* 28: 350-354.
- Fasehee H, Westers H, Bolhuis A, Antelmann H, Hecker M, Quax WJ, Mirolohi AF, van Dijnl JM, Ahmadian G (2011). Functional analysis of the sortase YhcS in *Bacillus subtilis*. *Proteomics* 11: 3905-3913.
- Gashtasbi F, Ahmadian G, Akbari K (2012). Immobilization of *Bacillus licheniformis* α -amylase on to bacillus subtilis spores. *12th Iranian Genetics Congress*, may 21-23, Tehran, Iran.
- Georgiou G, Stephens DL, Stathopoulos C, Poetschke HL, Mendenhall J, Earhart CF (1996). Display of β -lactamase on the *Escherichia coli* surface: outer membrane phenotypes conferred by Lpp'-OmpA'- β -lactamase fusions. *Protein Eng.* 9: 239-247.
- Jung HC, Kwon SJ, Pan JG (2006). Display of a thermostable lipase on the surface of a solvent-resistant bacterium, *Pseudomonas putida* GM730, and its applications in whole-cell biocatalysis. *BMC Biotechnol.* 6: 23.

- Hinc K, Ghandili S, Karbalaee G, Shali A, Noghabi KA, Ricca E, Ahmadian G (2010). Efficient binding of nickel ions to recombinant *Bacillus subtilis* spores. *Res Microbiol.* 161: 757-764.
- Hinc K, Isticato R, Dembek M, Karczewska J, Iwanicka A, Peszyńska-Sularz G, De Felice M, Obuchowski M, Ricca E (2010). Expression and display of UreA of *Helicobacter acinonychis* on the surface of *Bacillus subtilis* spores. *Microb Cell Fact.* 9: 2.
- Isticato R, Cangiano G, Tran HT, Ciabattini A, Medagliani D, Oggioni MR, De Felice M, Pozzi G, Ricca E (2001). Surface Display of Recombinant Proteins on *Bacillus subtilis* Spores. *J Bacteriol.* 183: 6294-6301.
- Kaufmann SHE (2001). How can immunology contribute to the control of Tuberculosis? *Nat Rev Immunol.* 1: 20-30.
- Kobayashi G, Toida J, Akamatsu T, Yamamoto H, Shida T, Sekiguchi J (2000). Accumulation of an artificial cell wall-binding lipase by *Bacillus subtilis* wprA and/or sigD mutants. *FEMS Microbiol Lett.* 2: 165-169.
- Kondo A, Ueda M (2004) Yeast cell-surface display-applications of molecular display. *Appl Microbiol Biotechnol.* 64: 28-40.
- Kuroda K, Shibasaki S, Ueda M, Tanaka A (2001). Cell surface engineered yeast displaying a histidine oligopeptide (hexa-His) has enhanced adsorption of and tolerance to heavy metal ions. *Appl Microbiol Biotechnol.* 57: 697-701.
- Kuroda K, Ueda M, Shibasaki S, Tanaka A (2002). Cell surface engineered yeast with ability to bind, and selfaggregate in response to, copper ion. *Appl Microbiol Biotechnol.* 59: 259-264.
- Kuroda K, Ueda M (2003). Bioadsorption of cadmium ion by cell surface-engineered yeasts displaying metallothionein and hexa-His. *Appl Microbiol Biotechnol.* 63: 182-186.
- Kuroda K, Ueda M (2006) Effective display of metallothionein tandem repeats on the bioadsorption of cadmium ion. *Appl Microbiol Biotechnol.* 70: 458-463.
- Kwon SJ, Jung HC, Pan JG (2007). Transgalactosylation in a Water-Solvent Biphasic Reaction System with β -Galactosidase Displayed on the Surfaces of *Bacillus subtilis* spores. *Appl Environ Microbiol.* 73: 2251-2256.
- Lang H, Mäki M, Rantakari A, Korhonen TK (2000). Characterization of adhesive epitopes with the OmpS display system. *Eur J Biochem.* 267: 163-170.
- Lee S Y, SutcliffeChoi J H, Xu Z (2003). Microbial cell-surface display. *Trends Biotechnol.* 21: 45-52.
- Matsumoto T, Fukuda H, Ueda M, Tanaka A, Kondo A (2002). Construction of Yeast Strains with High Cell Surface Lipase Activity by Using Novel Display Systems Based on the Flo1p Flocculation Functional Domain. *Appl Environ Microbiol.* 68: 4517-4522.
- Medagliani D, Ciabattini A, Spinosa MR, Maggi T, Marcotte H, Oggioni MR, Pozzi G (2001). Immunization with recombinant *Streptococcus gordonii* expressing tetanus toxin fragment C confers protection from lethal challenge in mice. *Vaccine* 19: 1931-1939.
- Mischo A, Wadle A, Wätzig K, Jäger D, Stockert E, Santiago D, Ritter G, Regitz E, Jäger E, Knuth A, Old L, Pfreundschuh M, Renner C (2003). Recombinant antigen expression on yeast surface (RAYS) for the detection of serological immune responses in cancer patients. *Cancer Immun.* 27: 3-5.
- Murai T, Ueda M, Yamamura M, Atomi H, Shibasaki Y, Kamasawa N, Osumi M, Amachi T, Tanaka A (1997). Construction of a Starch-Utilizing Yeast by Cell Surface Engineering. *Appl Environ Microbiol.* 63: 1362-1366.
- Narita J, Kenji O, Kitao T, Ishida S, Tomomitsu Sewaki, Sung M-H, Hideki F, Kondo A (2006). Display of α -Amylase on the Surface of *Lactobacillus casei* Cells by Use of the PgsA Anchor Protein, and Production of Lactic Acid from Starch. *Appl Environ Microbiol.* 72: 269-275.
- Nishitani T, Shimada M, Kuroda K, Ueda M (2010). Molecular design of yeast cell surface for adsorption and recovery of molybdenum, one of rare metals. *Appl Microbiol Biotechnol.* 86: 641-648.
- Rutherford N, Mourez M (2006). Surface display of proteins by Gram-negative bacterial autotransporters. *Microb Cell Fact.* 5: 22.
- Saffar B, Yakhchali B, Arbabi M (2005). Enhanced bioadsorption of cadmium and nickel by *E. coli* displaying a metal binding motif using CS3 fimbriae. *Iran J Biotechnol.* 3: 180-185.
- Samuelson P, Gunneriusson E, Nygren PA, Stahl S (2002). Display of proteins on bacteria. *J Biotechnol.* 96: 129-154.
- Shibasaki S, Maeda H, Ueda M (2009). Molecular Display Technology Using Yeast-Arming Technology. *Anal Sci.* 25: 41-49.
- Smith GP (1985). Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228: 1315-1317.
- Stover CK, Bansal GP, Hanson MS, Burlein JE, Palaszynski SR, Young JF, Koenig S, Young DB, Sadziene A, Barbour AG (1993). Protective immunity elicited by recombinant bacille Calmette-Guerin (BCG) expressing outer surface protein A (OspA) lipoprotein: a candidate Lyme disease vaccine. *J Exp Med.* 178: 197-209.
- Sutcliffe IC, Russell RR (1995). Lipoproteins of Gram-positive bacteria. *J Bacteriol.* 177: 1123-1128.
- Tafakori V, Ahmadian G, Amoozgar M (2012). metallothioneins and a chitin binding domain on *Escherichia coli* increase cadmium adsorption and cell. *Appl Biochem Biotechnol.* 167: 462-473 DOI: 10.1007/s12010-012-9684-x.
- Tavassoli S, Anchimiuk A, Nowakowska M, Hinc K, buchowski M, Ahmadian G (2012). Investigation of spore surface display of *Bacillus subtilis* β -galactosidase for developing a whole cell biocatalyst. *5th european spore conference.* London, U.K. 16-19 April.
- Tischer W, Kasche V (1999). Immobilized enzymes: crystals or carriers? *Trends Biotechnol.* 17: 326-335.
- Tsuchiya A, Kobayashi G, Yamamoto H, Sekiguchi J (1999). Production of a recombinant lipase artificially localized on the *Bacillus subtilis* cell surface. *FEMS Microbiol Lett.* 176: 373-378.
- Ueda M, Tanaka A (2000a). Genetic immobilization of proteins on the yeast cell surface. *Biotechnol Adv.* 18: 121-140.
- Ueda M, Tanaka A (2000b). Cell surface engineering of yeast construction of arming yeast with biocatalyst. *J Biosci Bioeng.* 90: 125-136.
- Ueda M, Tanaka A (2004) Yeast cell-surface display-applications of molecular display. *Appl Microbiol Biotechnol.* 64: 28-40.

- Vijayaraghavan K, Yun Y (2008). Bacterial biosorbents and biosorption. *Biotechnology Advances*. 26: 266-291.
- Wu CH, Ashok M, Chen W (2004). Versatile microbial surface-display for environmental remediation and biofuels production. *Trends Microbiol*. 16: 181-188.
- Wu ML, Chun Yung Tsai, Chen Tsai Hsia (2006). Cell surface display of Chi92 on Escherichia coli using ice nucleation protein for improved catalytic and antifungal activity. *FEMS Microbiol Lett*. 256: 119-125.
- Xu Z, Lee SY (1999). Display of polyhistidine peptides on the Escherichia coli cell surface by using outer membrane protein C as an anchoring motif. *Appl Environ Microbiol*. 65: 5142-5147.
- Xu Z, Bae W, Mulchandani A, Mehra RK, Chen W (2002). Heavy Metal Removal by Novel CBD-EC20 Sorbents Immobilized on Cellulose. *Biomacromolecules* 3: 462-465.
- Xu W, Huang M, Zhang Y, Yi X, Dong W, Gao X, Jia C (2011). Novel surface display system for heterogenous proteins on Lactobacillus plantarum. *Lett Appl Microbiol*. 53: 641-648.
- Zou W, Ueda M, Murai T, Tanaka A (2000). Establishment of a simple system to analyse the molecular interaction in the agglutination of Saccharomyces cerevisiae. *Yeast* 16: 995-1000.
- Zhu K, Chi Z, Li J, Zhang F, Li M, Yasoda HN, Wu L (2006). The surface display of haemolysin from Vibrio harveyi on yeast cells and their potential applications as live vaccine in marine fish. *Vaccine* 24: 6046-6052.

Archive of SID