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Cell Surface Display

Sharadwata Pan and Michael K. Danquah
Monash University
Australia

1. Introduction

The manipulation of the cell surfaces of prokaryotes (mainly bacteria) and eukaryotes (such as Yeast) has manifested to be an area of stupendous ongoing research, with intelligent widespread applications spanning different arenas of biological sciences (Charbit et al., 1988; Cruz et al., 2000; Francisco et al., 1993; Götz, 1990; Jostock & Dübel, 2005; Keskinan et al., 2004; Kotrba et al., 1999; Lee & Schnaitman, 1980; Liljeqvist et al., 1997; Martineau et al., 1991; Mizuno et al., 1983; Sousa et al., 1996; Taschner et al., 2002; Wernérus & Ståhl, 2004; Willett et al., 1995; Xu & Lee, 1999). Till date, majority of the surface display systems developed for Gram-negative bacteria involve introducing external peptides into surface-accessible loops of naturally displayed proteins. This sometimes put extreme size restrictions on the displayed components (Wernérus & Ståhl, 2004). However, this problem is more or less resolved since larger proteins could be inserted through some recently developed bacterial display systems for Gram-negative bacteria (Charbit et al., 1988; Cruz et al., 2000; Lee & Schnaitman, 1980; Mizuno et al., 1983; Xu & Lee, 1999). Thanks to some tireless research, it is now evident that the structural properties of the cell wall in Gram-positive bacteria, i.e. the thick peptidoglycan layer, make them suitable candidates for strict laboratory procedures and demanding field applications (Jostock & Dübel, 2005). On the other hand, lower transformation efficiency has been a significant disadvantage of using Gram-positive bacteria (Wernérus & Ståhl, 2004), considering if someone is working with surface-displayed conjugal libraries for affinity-based selections. However, libraries of significant size could also be obtained for Gram-positive bacteria. Transformation frequencies as high as $10^5 - 10^6$ colony forming units/ μg of DNA have been observed for *Staphylococcus carnosus* (Götz, 1990). Until recently, different surface displaying systems have been successfully developed (Lee et al., 2003). Based on their recombinant portfolios, these can be categorized into three principal groups: C-terminal fusion, N-terminal fusion, and Sandwich fusion. Natural occurring surface proteins with distinct restricting signals within their N-terminal part may use a C-terminal fusion mechanism to affix external peptides to the C terminus of that functional portion. In a similar way, a N-terminal fusion system points external proteins to the cell wall by using either *Staphylococcus aureus* protein A, fibronectin binding protein B, *Streptococcus pyogenes* fibrillar M protein, and *Saccharomyces cerevisiae* α -agglutinin, all of which contain C-terminal screening signals. However, in many surface proteins, the whole structure is an essentiality for successful aggregation, primarily because the anchoring regions are absent in their subunits (such as outer membrane proteins or OMPs). Here, the sandwich fusion plays a vital role. *Escherichia coli* PhoE, FimH, FliC, and PapA act as good carriers for sandwich fusion for small peptides (Xu & Lee, 1999).

Exhaustive investigations had been carried out in displaying antigens on the surface of different bacterial species that are not corresponding in structure or evolutionary origin (Charbit et al., 1988; Cruz et al., 2000; Francisco et al., 1993; Götz, 1990; Jostock & Dübel, 2005; Keskinan et al., 2004; Kotrba et al., 1999; Lee & Schnaitman, 1980; Liljeqvist et al., 1997; Martineau et al., 1991; Mizuno et al., 1983; Sousa et al., 1996; Taschner et al., 2002; Wernérus & Ståhl, 2004; Willett et al., 1995; Xu & Lee, 1999). The motive was to use them as carriers of vaccine-delivery, mainly for immunizations of or relating to mucous membranes. Several mechanisms have been developed to better the activated immunological response by mutual display of adhesins, mainly for targeting to the mucosal epithelium. Today, cheap whole-cell biocatalysts are a reality, thanks to the surface display of some enzymes on genetically engineered bacteria. Another emerging trend is the progressive use of display of metal-binding peptides on bacterial surfaces, resulting in efficient metal-binding capability. These recombinant bacteria may act as biosensors or in the quarantine of heavy metals in specialized bioremediation endeavors. So, it is now possible to synthesize ideal, conceptualized bacteria using these connecting strategies with increased specificity and affinity towards the target metal. This would result in significant usefulness of these types of bioadsorbents (Sousa et al., 1996). Also, a probable way of creating biofilters, biocatalysts or diagnostic devices is by effectual immobilization of these cells on solid supports. A summary of the microbial surface display systems has been done (see Table 1). So cell surface display as a mechanism has been accepted and applied for various biotechnological initiatives encompassing areas as important as vaccine delivery, bioremediation and selection platform (Wernérus & Ståhl, 2004), and an array of recent scientific findings indicate that it will continue to act as a promising tool for applied research in years to come.

2. Concepts and pre-existing surface display approaches

2.1 Surface display in Prokaryotes (gram-positive and gram-negative bacteria)

2.1.1 Gram-negative bacteria

Selection systems for the prokaryotes include cellular and phage display and are based on *E. coli*. This is because of its genetic build-up, culturing and maintenance protocols have been extensively studied and are pretty optimized with assuring reproducibility in laboratory and industrial scale. Majority of the outer membrane of *E. coli* is constituted of proteins, which epitomizes a range of adhering mechanisms for foreign sequences. The basic concept of surface display in gram-negative organisms is shown here (see Fig. 1) and some examples are summarized (see Table 2). Some of the common outer membrane proteins that have been used for surface display are (Jostock & Dübel, 2005):

LamB: LamB gene encodes the outer membrane protein maltoporin of *E. coli* which facilitates the transfer of maltose and maltodextrin across the outer membrane. A large polypeptide library of around 5 million different clones uses Maltoporin as the carrier protein. Metal-identifying polypeptides have been isolated by displaying this library on *E. coli* and selecting on metals such as Gold or Chromium.

OmpT: It is an important member of the Omptin family of proteases that has been surface-displayed in *E. coli*. *E. coli* cells that express effective OmpT could be augmented from cells expressing non-effective OmpT by nearly 5000-fold in a single round by coupling both Fluorescence Activated Cell Sorting (FACS) and Fluorescence Resonance Energy Transfer (FRET). For developing enzymes, the same selection principle has been used.

Lpp-OmpA: It has been used extensively for displaying antigens, antibodies, peptides and enzymes. The Lpp-OmpA system is a combination of Lpp (the first nine N-terminal amino

Carrier protein	Host Organism	Insert size	Fusion	Insert
<i>Prokaryotes</i>				
Gram-negative				
FimH	<i>E.coli</i>	7–52 aa	Intern	Peptide library
Flagellae	<i>E.coli</i>	11–302 aa	Intern	Peptide library epitope mapping
Pilin	<i>E.coli</i>	7–56 aa	Intern	Peptide epitopes
Intimin	<i>E.coli</i>	128 aa	C-terminal	Gene-fragment peptide library
Invasin	<i>E.coli</i>	18 aa	C-terminal	Peptide library
LamB	<i>E.coli</i>	11-232 aa	Intern	Peptide library
OmpC	<i>E.coli</i>	162 aa	Intern	Peptides
PAL	<i>E.coli</i>	ca. 250 aa	N-terminal	scFv fragments
Gram-positive				
Protein A	<i>S. carnosus</i> / <i>S. xylosus</i>	ca. 250 aa	N-terminal N-terminal	scFv fragments
Protein A	<i>S. carnosus</i>	Up to 397 aa	N-terminal	Cellulose binding domain
<i>Eukaryotes</i>				
α -agglutinin receptor	Yeast	Up to 620 aa	C-terminal	MHC Class I and II Cytokines Growth Factors Selectines

Table 1. Some surface display systems in both prokaryotes and eukaryotes that are suitable for the functional screening of molecular aggregation. Here ‘aa’ symbolizes amino acids. Reproduced from an earlier review (Jostock & Dübel, 2005).

acids and the signal sequence)and an OmpA fragment of the original protein (containing five of the eight membrane covering loops). For displaying on the outer membrane of *E. coli*, heterologous proteins (up to 40 kilodaltons or kDa) can be blended to the C-terminus of the Lpp-OmpA fusion protein. This is also a convenient method for displaying the target antigen.

Inp: A glycosylphosphatidylinositol (GPI)-anchor sequence is responsible for binding the Ice-nucleation protein (Inp) of *Pseudomonas syringae* to the surface of the cell. By this way, it can be used as a carrier (in an effective form) to display enzymes on the surface of *E. coli*. The fact that single-chain antibodies (scFVs) have already been displayed as Inp-fusion proteins on *E. coli* makes this system tailor-made for surface displaying antibody libraries.

Intimin: Adhesins (like Intimin) are expressed by *E. coli* strains (capable for causing diseases in the intestinal tract)on their surfaces. This particularly connects with the destined structures on the host cells (eukaryotic). Coalition partners of up to 128 aa residues, derived from different species, have been practically displayed on *E. coli* K-12 strain surface by displacing the two carboxyterminal domains of the EaeA intimin of *E. coli* O157:H7. A common estimation is that one cell displays around 35 thousand shortened intimin molecules.

FimH: Type 1 fimbriae are a common surface feature of majority of *E. coli* strains. FimH, which can be found on the apex of type 1 fimbriae, helps in binding to sturctures that contain α -D-mannose. Without manipulating the biological function of fimbriae, special sequences (from different species) can be inserted in the C-terminal part of FimH. Scientists have selected

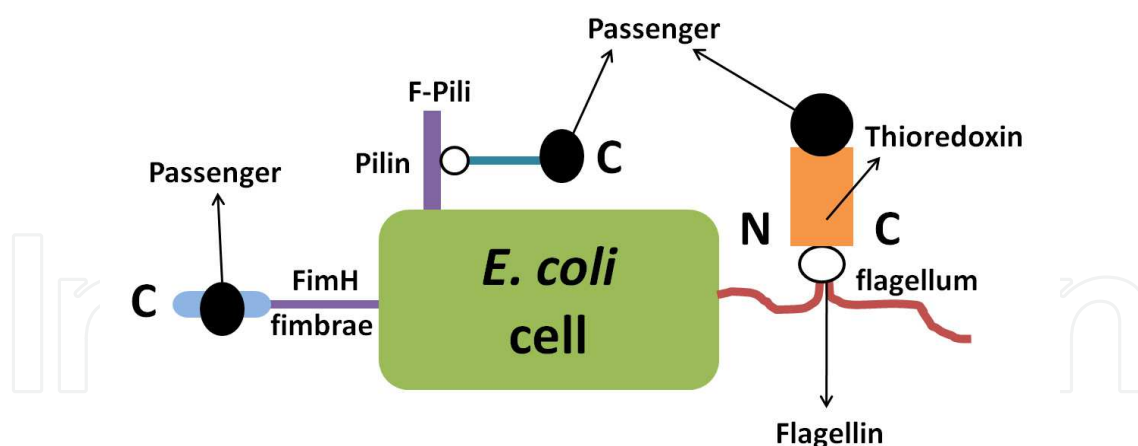


Fig. 1. Cell organelle associated surface display mechanism in Gram negative bacteria. Reproduced and redrawn from (Jostock & Dübel, 2005).

for Ni^{2+} binding clones by constructing an arbitrary peptide library in the FimH protein that can be displayed on *E. coli*.

Invasin: For displaying a peptide library on *E. coli*, a carrier protein in the form of Invasin (of *Y. pseudotuberculosis*), has been in practice. The C-terminal part of invasin binds to integrins and can be displaced by arbitrary peptides (with ten subunits). Peptides with cell binding capabilities may be isolated by library screening on whole mammalian cells.

2.1.2 Gram-positive bacteria

There has been an abundant use of gram positive bacteria in presenting fragments of proteins from different species (between 15 and 459 amino acid residues) (Jostock & Dübel, 2005). However, these applications are cornered to the area of vaccine production, due to the immunological relevance of these gram-positive bacterial strains. Further, a notion of non-trustworthiness prevails in the wider community citing the non-optimization of genetic manipulation of some of these bacteria, as opposite to the scenario in *E. coli*. Genetically altered expression and secretion systems (for proteins) in *Bacillus subtilis* and many other gram positive bacteria are common today (Götz, 1990; Liljeqvist et al., 1997; Wernérus & Ståhl, 2004). The concept of surface display in such organisms is shown here (see Fig. 2) and some examples are summarized (see Table 3).

Till date, the expression of single chain antibodies as fusions to *Staphylococcus aureus* Protein A (SpA) on the non-harmful and food-grade *S. xylosus* and *S. carnosus* strains (Wernérus & Ståhl, 2004) indicates the suitability of Staphylococcal cells as candidates for selecting antibody repositories. However, due to the lower transformation efficiency (as compared to *E. coli*), the use of *Staphylococci* as hosts for conjunctural libraries has suffered. The primary reason being the limitation of the library-size that could be obtained (Wernérus & Ståhl, 2004).

2.2 Surface display in Eukaryotes (Yeast)

The surface display system in yeast demonstrates a C-terminal attachment to the Aga2p subunit of *Saccharomyces cerevisiae* α -agglutinin receptor. This is bound to the Aga1p subunit through two disulphide bonds, which is attached to the β -glucan of the cell wall via covalent bonds. This system has been authenticated for displaying antibody fragments (including Fab fragments), peptides and other protein domains (Jostock & Dübel, 2005). There is large degree

Display system	Displayed protein
<i>Category: Outer Membrane Proteins (OMPs)</i>	
OmpA	Peptides, Malarial antigens
LamB	C3 epitope of poliovirus
	Peptide library
	Peptides
OprF	Malaria epitope
PhoE	Part of FMDV
OmpS	Epitopes
OmpC	(His) ₁₆₂
FhuA and BtuB	T7 tag, myc epitope
Lpp'OmpA	Green Fluorescent Protein
	β -lactamase
	PhoA
Invasin	Peptide libraries
EaeA Intimin	Epitope mapping
Inp	CM Cellulose
	Salmobin
	OPH (library)
<i>Category: Autotransporters</i>	
IgA β	CTB, MT
AIDA-I	CTB and peptide antigen
	β -lactamase
Ag43	FimH lectin domain
MisL	Malaria epitope
<i>Other systems</i>	
Peptidoglycan associated lipoprotein	Antibody fragments
TraT	Poliovirus epitope
Pullulanase	β -lactamase

Table 2. Selective examples where Gram-positive bacteria have been used for surface-display applications. Reproduced from an earlier review (Wern rus & St hl, 2004).

of similarity between the analysis and selection of yeast displayed libraries to that of bacteria. Healthy, boisterous systems are also a reality (Boder & Wittrup, 1997; Murai et al., 1998; Sousa et al., 1998). The concept of surface display is nicely elaborated in an earlier work (Jostock & D bel, 2005).

3. Some novel applications of cell surface display technique

Till date, there had been many significant contributions in the area of cell surface display of heterologous proteins. Some of them are categorized into common application areas (see Table 7) and briefly described below, mainly to get an idea of the wide applicability of the surface display technique. Selected examples from an earlier review have been summarized (see Tables 4–6).

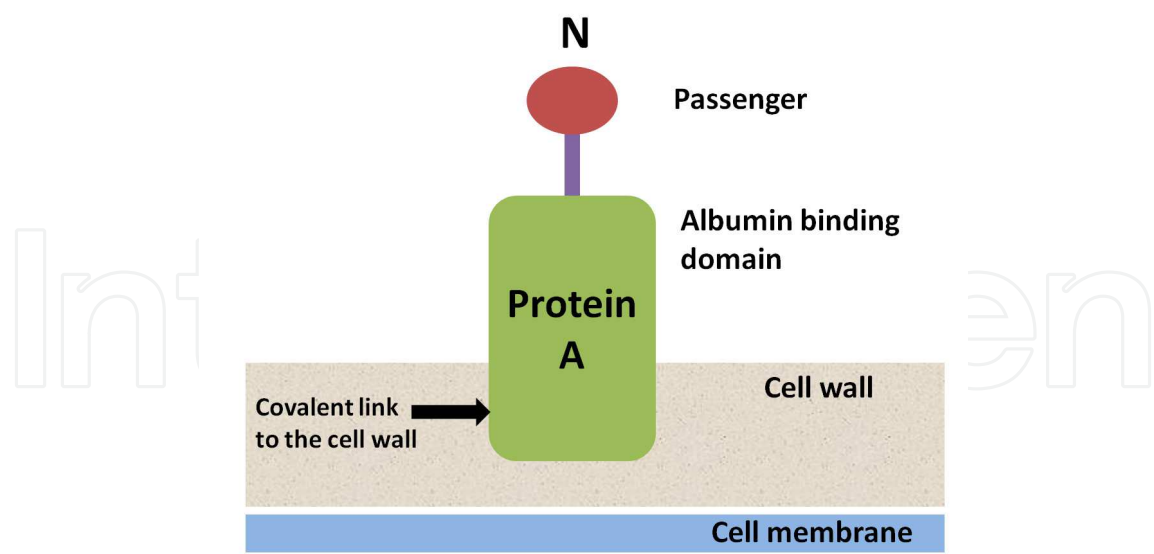


Fig. 2. Cell surface display in Gram positive bacteria. *S. aureus* protein A serves as fusion partner for the surface display. Reproduced and redrawn from (Jostock & Dübel, 2005).

Display system	Displayed protein
Protein A	scFv
	RSV G-protein
	IgA- and IgE-specific affibodies
	Polyhistidyl peptides
	Streptavidin
FnBPB	<i>Staphylococcus hyicus</i> lipase, β -lactamase
M6	E7 protein of human papillomavirus
	White-faced-hornet (<i>Vespula maculata</i>) antigen
	Tetanus toxin fragment C
	Staphylococcal nuclease
SpaPI	<i>Bordella pertussis</i> SI subunit
CwbA	<i>Yersinia pseudotuberculosis</i> invasin
CotB	Tetanus toxin
Mtb19	OspA lipoprotein from <i>Borrelia burgdorferi</i>
SLH	Tetanus toxin fragment C

Table 3. Selective examples where Gram-positive bacteria have been used for surface-display applications. Reproduced from an earlier review (Wernérus & Ståhl, 2004).

3.1 Vaccine delivery and diagnostic devices

Charbit et al. (1988) demonstrated the expertise of a vector for expressing external polypeptides on the surface of *E.coli*. Their work has formidable potential to create applications. This includes production of an efficient live bacterial vaccine. Liljeqvist et al. (1997) had expressed cholera toxin B subunit (CTB) from *Vibro cholerae* on the surface of two staphylococcal species, *Staphylococcus xylosus* and *Staphylococcus carnosus*. Their work showed enough promise for designing live vaccine delivery systems in bacteria (through the mucosal pathway). According to the authors, further work can be carried out in this area. Rockberg

et al. (2008) introduced a remarkable antibody-identified mapping method for epitopes. They expressed antigenic fragments on bacteria and followed it up with antibody-dependent sorting through flow-cytometry. The authors proved that epitope-specific antibodies may be synthesized using bacteria cells. Dou et al. (2009) used surface display technology in bacteria and investigated the pathogenicity of the Japanese Encephalitis Virus (JEV). The authors achieved this by constructing a genetically manipulated *Salmonella typhimurium* BRD509 strain and surface-displayed domain III of the covering protein of the JEV (JEDIII) with the aminoterminal domain of the ice nucleation protein (INPN). They used Western blot and immunohistochemical staining to confirm the surface display. According to the authors, it is now feasible to study the pathogenesis of JEV using their approach. In a recent work, phage display technology has been utilized by Urushibata et al. (2010) to bind antigen-binding (Fab) fragments and single chain variable fragments (scFv) to staphylococcal enterotoxin B (SEB) protein. Their work is noted for developing a unique method for preparing an anti-SEB Fab fragment library. The usefulness of these agents as molecular recognition tools was confirmed by successful application to the SEB determinants from serum by Western blotting. The authors conclude that SEB can be identified by their synthesized scFv and this can even replace anti-SEB immunoglobulins as a cost-effective SEB identification tool.

3.2 Enzymes and biocatalysis

Murai et al. (1998) showed that a yeast cell, which is surface-manipulated with enzymes (alpha-glucosidase and carboxymethylcellulase), acquire the ability to digest cellooligosaccharides. According to the authors, this can be the initiation of the digestion of cellulosic substances by *S. cerevisiae* that expresses cellulase genes from different species. As evident from the conclusion of this work, this can be further researched for identifying the next digestion steps. Tsai et al. (2009) through a contemporary work, showed that a single yeast strain containing the required cellulolytic enzymes: two endoglucanases and one exoglucanase (through a displayed minicellulosome) can actively carry out both concurrent and cooperative saccharification and fermentation of cellulose to ethanol. The authors conclude that their overall yield was 0.49 gram of ethanol produced per gram of carbohydrate consumed, which corresponds to 95% of the theoretical value.

3.3 Biosensors and bioadsorbents

Sousa et al. (1996) had displayed poly-His peptides and shown increased adsorption of metals by bacterial cells. From the work, it can be concluded that by expressing poly-His peptides, bacteria may act as adsorbents having metal affinity. Now, it is possible to engineer microorganisms which may facilitate bioadsorption of heavy metal ions. According to the authors, exquisite research opportunities exist for professionals in this particular area. In another interesting work, Sousa et al. (1998) showed that Yeast (CUP1) and mammalian (HMT-1A) metallothioneins can be effectively expressed in *E. coli* as attachments to LamB protein. The authors have clearly demonstrated that these hybrid proteins can be expressed. This has enhanced the natural capability of *E. coli* cells to bind Cd^{2+} ions to about 15 – 20 fold.

3.4 Selection platform

Martineau et al. (1991) developed a method to derive and analyze anti-peptide antibodies without actually synthesizing peptides. The peptide of choice was expressed by them as a genetical insert within two separate receiver bacterial proteins (MalE and the LamB proteins from *E. coli*). According to the authors, more work can be done in this frontier. In another

Display system	Organism	Displayed antigen	Animal model	Results
<i>Gram-negative</i>				
MisL	<i>S. typhimurium</i>	Malarial (NANP)	Mice	Ag-specific IgG
LamB	<i>E. coli</i>	HbsAg (preS2)	Mice and rabbits (i.v.)	Ag-specific IgG
	<i>E. coli</i>	Polio epitope (C3)	Mice (i.p.)	Ag-specific IgG and IgM
OmpA	<i>S. typhimurium</i>	Malarial epitopes (SERP and HRPII)	Mice (orally)	Ag-specific IgG and IgM
Chimaeric OmpA	<i>S. typhimurium</i>	Malarial epitope (M3)	Mice (i.p.)	Ag-specific IgG
<i>Gram-positive</i>				
SpA	<i>S. xylosus</i>	RSV antigen	Mice (orally)	Ag-specific IgG
	<i>S. carnosus</i>	Streptococcal protein G/CTB	Mice (i.n.)	Ag-specific IgG and IgM
	<i>S. carnosus</i>	CTB/RSV	Mice (i.n.)	Protection
M6	<i>S. gordonii</i>	TTFC	Mice (i.n. and subcut.)	Protection
		LTB and HIV-I epitope V3	Mice (subcut.)	Ag-specific IgG
SpaPI	<i>S. gordonii</i>	PTS SI	Mice (i.p.)	Protection
		PTS SI	Mice (orally)	Ag-specific sIgA
SLH	<i>B. anthracis</i>	TTFC	Mice (subcut.)	Protection
CotB	<i>B. subtilis</i>	TTFC	Mice (subcut.)	Ag-specific IgG
Lipoprotein Mtb19	<i>M. bovis</i> -BCG	OspA from <i>B. burgdorferi</i>	Mice (i.n.)	Ag-specific IgG and sIgA
Abbreviations: i.d., intradermal; i.n., intranasally; i.p., intraperitoneally; i.v., intravenously; subcut., subcutaneous; Ag., Antigen; PTS, Pertussis Toxin Subunit				

Table 4. Selected examples, where live bacteria with surface displayed antigens have been used as vaccine delivery vehicles. Reproduced from an earlier review (Wernérus & Ståhl, 2004).

Display system	Displayed protein
<i>Gram-negative</i>	
Pullulanase	β -lactamase
Lpp'OmpA	β -lactamase
Inp	<i>Zymomonas mobilis</i> levansucrase (LevU)
	<i>Bacillus subtilis</i> CM-cellulose
	Salmobin
AIDA-I	β -lactamase
Inp and Lpp'OmpA	OPH and CBD
<i>Gram-positive</i>	
FnBPB	<i>S. hyicus</i> lipase and β -lactamase

Table 5. Selected examples of functionally active enzymes displayed on bacteria. Reproduced from an earlier review (Wernérus & Ståhl, 2004).

wrok, a single chain antibody fragment (scFv), containing the variable heavy and variable light regions from two different monoclonal antibodies had been expressed on the outer

Display system	Displayed protein	Strain
Lpp'OmpA	MT	<i>E. coli</i>
LamB	MT (mammalian/yeast)	<i>E. coli</i>
LamB	MT (α -domain)	<i>E. coli</i>
IgA β	MT (mouse)	<i>Pseudomonas putida</i>
Lpp'OmpA	PC (synthetic)	<i>E. coli</i>
Inp	PC (synthetic)	<i>Maraxella sp.</i>
SpA	(His) ₆	<i>S. carnosus</i> / <i>S. xylosus</i>
LamB	(His) ₆	<i>E. coli</i>
OmpC	(His ₆) ₁₂	<i>E. coli</i>
LamB	HP / CP	<i>E. coli</i>
OmpA	HSQKVF	<i>E. coli</i>
SpA	Engineered CBD	<i>S. carnosus</i>
FimH	Peptide library	<i>E. coli</i>

Table 6. Selected examples, where metal-binding peptides and proteins have been expressed on the surface of bacteria for environmental applications. Reproduced from an earlier review (Wern rus & St hl, 2004). Here ‘MT’ stands for Metallothioneins and ‘PC’ stands for Phytochelatins.

surface of *E. coli* (Francisco et al., 1993). The high level expression of this scFv attachment was shown to bind the hapten with increased compatibility and particularity. Boder & Wittrup (1997) had shown that for manipulating cytokines, antibodies and receptors, display on the cell wall of yeast may be a suitable strategy. However, for effective folding and activity, post translational modification has to be a characteristic of the endoplasmic reticulum. The authors conclude that through this work, kinetic parameters can be distinguished for protein binding to soluble ligands through flow cytometry. Hoischen et al. (2002) showed that external proteins in the cytoplasmic membrane of *E. coli* and *Proteus mirabilis* can be fixed using an ingenious surface display strategy of the membrane. These bacterial strains are steady and lack cell walls. They had fused the reporter protein, staphylokinase (Sak) to the membrane-spanning regions of some fundamental membrane proteins from these organisms. The authors confirm that accumulation of the fusion proteins (that are strongly attached to the cytoplasmic membrane) is not a common phenomenon. It is also reported that the protein was confined on the external surface. According to the authors, this technique may generate various application areas which may revolutionize the range of applications of surface display systems. Bessette et al. (2004) demonstrated that it is possible to bind briskly segregated peptides to promptly selected targets with high compatibility. The authors synthesized and screened a large library for binding to some unrelated proteins. These included targets which were previously used in phage display selections like human serum albumin, human C-reactive protein etc. According to the authors, this efficient procedure should be helpful in lot of applications concerning molecular identification since it identifies reagents for peptide affinity. Zahnd et al. (2007) came up with a fascinating gradual procedure to display ribosome selection employing an *E. coli* S30 extract for *in vitro* protein synthesis. The authors agree that in ribosome display, the library range is not restricted by the efficiency of transformation of the bacterial cells. Rather, it is limited by the number of distinct ribosomal complexes that are present in the reaction volume. This dissimilarity is actually the number of ribosomal complexes that show a functional protein. The authors also present a procedure that displays ribosomes through eukaryotic *in vitro* machinery for protein synthesis. Kenrick & Daugherty

Surface displayed proteins	Application area for recombinant bacteria
Antibody fragments	Diagnostic devices
Enzymes	Whole Cell Biocatalysis
Adhesins and antigens	Vaccine delivery
Metal binding peptides	Biosensors and Bioadsorbents
Antibody and peptide libraries	Selection platform

Table 7. Examples of surface displayed proteins and possible application areas for recombinant bacteria (Wernérus & Ståhl, 2004).

(2010) demonstrated an analytical extracting process for affinity maturing ligands with particular given targets. These targets are displayed on the external surface of *E. coli*. By using flow cytometric analysis (involving several parameters), the authors conclude that bacterial surface display proves to be a novel and significant mechanism for the discovery and optimization of peptide ligands that are specific to a particular protein.

4. Concluding remarks

It is now evident that till today, an array of proteins derived from different species have been targeted and expressed on the cell surfaces of Gram-negative or Gram-positive bacteria, and a number of different application areas have been identified. Bacterial surface display will be a continuously growing research area and both Gram-negative and Gram-positive bacteria of various kinds will be thoroughly investigated for different biotechnological applications in the near future. Though several surface display techniques have been developed till date, problems do exist and will continue to haunt researchers. Quality of the peptide library displayed on cell surface and reduced enzyme activity while developing whole-cell biocatalysts are now recognized issues. Another significant challenge is the surface display of multiple proteins or proteins consisting of more than one subunit, which tends to make the cells weak and in some cases, may lead to fatality. However, the ultimate challenge remains the transformation of the numerous laboratory-scale successes in this area to the level industrial productivity. With smarter technologies available, this will happen sooner or later, especially in the areas of bioconversion and peptide library screening. Hopefully, this will pave the way for even more successful commercial applications of cell surface display.

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Biodiversity is strongly affected by the rapid and accelerating changes in the global climate, which largely stem from human activity. Anthropogenic activities are causing highly influential impacts on species persistence. The sustained environmental change wildlife is experiencing may surpass the capacity of developmental, genetic, and demographic mechanisms that populations have developed to deal with these alterations. How biodiversity is perceived and maintained affects ecosystem functioning as well as how the goods and services that ecosystems provide to humans can be used. Recognizing biodiversity is essential to preserve wildlife. Furthermore, the measure, management and protection of ecosystem biodiversity requires different and innovative approaches. For all these reasons, the aim of the present book is to give an up-to-date overview of the studies on biodiversity at all levels, in order to better understand the dynamics and the mechanisms at the basis of the richness of life forms both in terrestrial (including agro-ecosystems) and marine environments.

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University Campus STeP Ri
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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