Review

An overview of yeast cell wall proteins and their contribution in yeast display system

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Abstract: Yeast surface display has become an increasingly popular tool for protein engineering and library screening applications. Although, recent advances have greatly expanded the capability of yeast surface display, the protein display system is still far away from industrial application. One of the major components of a stable, efficient and successful yeast surface display system is cell wall anchor protein with which our desired foreign protein will be attached. We studied 80 different yeast cell wall anchored proteins originated mostly from Saccharomyces cerevisiae and Candida albicans. We studied in details all the cell wall proteins in order to find out suitable cell wall proteins to recommend for the researchers to use in the construction of yeast display system. We considered selective physical properties of different yeast cell wall proteins that are crucial for selecting best suited cell surface anchor proteins which are molecular weight, binding domain of anchor protein, length of amino acid and fusion site. Finally, our studies showed that Ccw11, Ccw12, Cwp1, Cwp2, Dan1, Gas1, Gas5, Exg1, Ycr89, Ecm33, Pga4, Sap9, Sap10, Pst1, Pir1, Pir2, Pir3, Pir4, Cis1, Scw4, Scw6, Bgl2, Uth1, Scw1 are the promising and suitable cell wall anchor proteins could be used in construction of yeast cell surface display system. Additionally, this review presents detailed information about all the cell wall proteins in a single work. The future researchers in this field will be able to construct more efficient yeast display system for recombinant protein production at industrial scale using the knowledge presented in this work.

Keywords: yeast cell wall protein; yeast display system; GPI anchored protein; Pir protein; recombinant protein production

1. Introduction

Surface display of proteins by incorporation into the cell walls of different microorganisms is a method established in the last two decades as a promising way of creation of new tools for modern biotechnology. Both bacterial and yeast cells have been used for this purpose. Among all the strains used to construct yeast cell surface display system, Saccharomyces cerevisiae and Candida albicans are the best studied and characterized till now. Although a number of potential biotechnological applications of microbial surface-displayed proteins have been reported in the last two decades, it is still challenging how to improve the efficiency of display of protein complexes or cofactor containing enzymes or co-display of multiple enzymes and increase the quantity of protein displayed on the yeast surface.

Yeast cell wall proteins can be classified according to their bonding approaches to the cell wall. Some proteins (SCWs) are attached to β-1, 3-glucan chain non-covalently, mainly by hydrogen bonding (Levin, 2011). Other type of proteins is bounded through covalent bonding which consist of both GPI-anchored proteins and Pir (Proteins with Internal Repeats) proteins. GPI-anchored proteins comprise the largest group of yeast cell wall protein. These are covalently attached cell wall proteins linked to β-1, 3-glucan through the remnants of glycosylphosphatidylinositol (GPI) anchors and β-1, 6-glucan (Klis et al., 2006.). Other group of covalently linked cell wall proteins are Pir proteins which are linked to β-1,3-glucan through alkali labile ester linkages.
Based on these binding approaches of cell wall proteins, the surface display systems in the yeast could be classified into three categories: Flo1 system, GPI system and Pir system. Flo1-based display system uses a lectin like cell wall protein Flo1 as anchor. The Flo1 anchor system provides C- or N-terminal terminal fusion. Pir proteins (Pir1-4) are family of covalently linked cell wall proteins characterized by conserved repetitive units, provides two options to fuse with the target proteins: C-terminal and inserted fusion. GPI proteins are linked to the β-1, 6-glucan of the cell wall by the remnant of C-terminal GPI-anchor and can provide only N-terminal display of heterologous proteins.

Yeast display system based on the expression of translational fusion of a cell wall anchoring protein and the desired target protein. Although, lots of yeast cell wall proteins are identified, plenty of options are open in order to select cell wall anchored protein which will strongly immobilize the targeted protein on the cell surface without interference with the stability or activity of the displayed protein. Fusion of this protein of interest could be done either the C-terminal end or the N-terminal end of the cell wall anchored protein. Selecting the appropriate cell wall anchoring protein is the crucial step for performing this total experiment. None of the reports summarized all the yeast cell wall proteins identified till now in a single place. That’s why; we have summarized 80 different cell wall proteins in this study so far. Several of them have been used successfully as anchor proteins in the last two decades.

There are some parameters such as bonding approaches of the cell wall protein; molecular weight; length of amino acids chain; binding site etc. that are crucial for selecting specific cell wall protein for construction of an efficient yeast display system. Generally most extensively used cell wall proteins in yeast surface display system are proteins that are covalently bound to the cell wall. Because this type of bonding gives strong attachment of the protein to the cell wall and facilitates the ease of quantification as well as prevent unnecessary wash-out during later treatment. Other important parameter to be selected is the molecular weight of the protein. Usually medium sized and molecular weighted proteins are preferable. Very high weighted proteins can be problematic as well as low weighted protein should be avoided. Binding site of the protein must be determined for ensuring the effectiveness of the protein. Catalytic domain and binding site are two important sites to be addressed. If the protein’s binding site is present close to the catalytic domain then it can directly affect the process of catalysis. In this study, we found many yeast cell wall proteins that were unused for constructing yeast surface display system. Many of them play important role in the cell wall stability and integrity. But the main problem is that, in most of the cases their fusion site or binding domain is not identified so far. If all the parameters and necessary features for anchor proteins can be identified, then these proteins will be used in yeast surface display system. Yeast surface display system also faces difficulties with insufficient documentation of all identified yeast cell wall proteins in a single place. In this review, we tried to collect all the necessary information about these anchoring proteins from various reliable sources and organized and presented in a single platform.

2. Literature review
2.1. Architecture of yeast cell wall
Yeast cell wall consists of about 85% of polysaccharides and 15% proteins but the relative amount of cell wall components can vary depending on yeast species, growth conditions and stress (Teparić and Mrša, 2013). The cell wall of S. cerevisiae is 110–200 nm wide (Dupres et al., 2010; Yamaguchi et al., 2011) and forms a bilayered structure (Figure 1) composed of an internal skeletal layer of glucan and a fibrillar brush-like outer layer (Osumi, 2012). The internal layer is composed of β-1,3- and β-1,6-glucan while the outer layer is composed predominantly of mannoproteins (Shibasaki et al., 2009). Mannoproteins are linked to β-1,6-glucose chains through a glycosylphosphatidylinositol (GPI) anchor or to β-1,3-glucan through an alkali-labile bond (Yamaguchi et al., 2011; Teparić and Mrša, 2013). Chains of β-1,3-linked glucose residues are branched by β-1,6 linkages forming a fibrillar β-1,3-glucan. The fibrillar β-1,3-glucan serves as a backbone to which chitin, β-1,6-glucan, and some mannoproteins are linked.
2.2. Yeast cell wall proteins

Different types of cell wall proteins (CWPs) have been identified in several yeast species. Most of them are found in outer cell wall membrane. GPI-anchored protein and Pir proteins constitute the major classes which are covalently linked cell wall proteins. Other group comprises several members which are Non-covalently bounded CWPs (Figure 1).

2.2.1. Covalently linked cell wall proteins

2.2.1.1. GPI-anchored proteins

More than 70 different GPI anchored proteins have been identified in different yeast species so far. It is estimated that half of them reside in the cell wall and most of them are connected to the β-1, 6-glucan chain by covalent linkages. Glycosylphosphatidylinositol (GPI) anchored proteins, are directed through the secretory pathway to the extracellular face of the plasma membrane by lipid anchors at their C terminal. GPI-anchored proteins destined for the cell wall are liberated from the plasma membrane by cleavage of their anchors. Then this lipidless GPI remnants of GPI–CWPs become linked to the external surface of the β-1, 3-glucan network indirectly through β-1, 6-glucan chains. This proteins are connected through covalent linkage. The enzyme-phospholipase C is used to cleave this bond.

2.2.1.2. Pir proteins

The other major class of CWPs is known as Pir protein that is represented by five related polypeptides, Pir 1-5. This Pir proteins are attached directly to β-1,3-glucan chains through a linkage that involves their repeat sequences, DGGφQ (where φ is any hydrophobic residue). The glucan chain is linked to the protein through the γ-carboxyl group of a Glu residue (within the repetitive sequence) evidently produced through a transglutaminase-type reaction that converts the first Gln residue to Glu. Most of them contain several repeat sequences that may provide sites for cross-linking of multiple β-1,3-glucan chains. This class of proteins are distributed throughout the inner glucan network, consistent with their attachment to β-1,3-glucan. These are covalently linked cell wall proteins which can be cleaved by mild alkali treatment (30 mM NaOH at 4°C) or by the treatment with β-1,3-glucanase enzyme.

Major covalently linked yeast cell wall proteins are mentioned with different important properties and functions in Table 1.

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**Figure 1. Architecture of yeast cell wall.**
## Table 1. List of covalently linked yeast cell wall proteins.

| Protein name | Molecular weight (KDa) | Length (a.a.) | Types of binding | Site of protein display | Functions (role in cell wall organization) | References |
|--------------|------------------------|---------------|------------------|------------------------|------------------------------------------|------------|
| Aga1         | 73                     | 725           | GPI anchor protein| N-terminal display     | Involved in agglutination during mating.  | Teparić et al., 2010 |
| Aga2         | 9                      | 87            | GPI anchor protein| N-terminal display     | Involved in agglutination during mating.  | Teparić et al., 2010 |
| Agaα1        | 250                    | -             | GPI anchor protein| N-terminal display     | Involved in agglutination during mating.  | Teparić et al., 2010 |
| Sed1         | 34                     | 338           | GPI anchor protein| N-terminal display     | Involved in lytic enzyme resistance.     | Teparić et al., 2010 |
| Flo1         | 161                    | 1537          | GPI anchor protein| N-terminal display     | Involved in flocculation.                | Debra et al., 2015  |
| Flo5         | 112                    | 1075          | GPI anchor protein| N-terminal display     | Involved in flocculation.                | Debra et al., 2015  |
| Flo9         | 138                    | 1322          | GPI anchor protein| N-terminal display     | Involved in flocculation.                | Debra et al., 2015  |
| Flo10        | 122                    | 1169          | GPI anchor protein| N-terminal display     | Involved in flocculation.                | Debra et al., 2015  |
| Flo11        | 136                    | 1367          | GPI anchor protein| N-terminal display     | Involved in flocculation and biofilm formation. | Debra et al., 2015  |
| Ccw12        | 13                     | 133           | GPI anchor protein| N-terminal display     | Plays a role in maintenance of newly synthesized areas of cell wall. | Ragni et al., 2007b |
| Ccw14        | 23                     | 238           | GPI anchor protein| N-terminal display     | Electrophoretic mobility of phosphorylated wall components of *Saccharomyces cerevisiae*. | Teparić et al., 2010 |
| Ccw22        | 14                     | 135           | GPI anchor protein| N-terminal display     | Specific biological function is unknown. |                        |
| Sag1         | 70                     | 650           | GPI anchor protein| N-terminal display     | Helps in sexual agglutination.           | Teparić et al., 2010 |
| Cwp1         | 24                     | 239           | GPI anchor protein| N-terminal display     | Plays a role in stabilizing the cell wall. | Smits et al., 2006  |
| Cwp2         | 9                      | 92            | GPI anchor protein| N-terminal display     | Plays a role in stabilizing the cell wall. | Smits et al., 2006  |
| Tir1         | 25                     | 254           | GPI anchor protein| N-terminal display     | Anaerobiosis.                           | Teparić et al., 2010 |
| Tir2         | 25                     | 251           | GPI anchor protein| N-terminal display     | Anaerobiosis.                           | Teparić et al., 2010 |
| Tir3         | 26                     | 269           | GPI anchor protein| N-terminal display     | Anaerobiosis.                           | Teparić et al., 2010 |
| Tir4         | 48                     | 487           | GPI anchor protein| N-terminal display     | Anaerobiosis/Tir4 sterol uptake.         | Teparić et al., 2010 |
| Tip1         | 21                     | 210           | GPI anchor protein| N-terminal display     | Anaerobiosis.                           | Teparić et al., 2010 |
| Dan1         | 30                     | 298           | GPI anchor protein| N-terminal display     | Anaerobiosis/sterol uptake.             | Teparić et al., 2010 |
| Dan2         | 13                     | 124           | GPI anchor protein| N-terminal display     | Anaerobiosis/sterol uptake.             | Teparić et al., 2010 |
| Dan3         | 13                     | 120           | GPI anchor protein| N-terminal display     | Anaerobiosis/sterol uptake.             | Teparić et al., 2010 |
| Fit1         | 52                     | 528           | GPI anchor protein| N-terminal display     | Facilitator of iron transport.           | Teparić et al., 2010 |
| Fit2         | 15                     | 153           | GPI anchor protein| N-terminal display     | Facilitator of iron transport.           | Teparić et al., 2010 |
| Fit3         | 20                     | 204           | GPI anchor protein| N-terminal display     | Facilitator of iron transport.           | Teparić et al., 2010 |
| Gene | ID | MIPS | GPI anchor protein | N-terminal display | Function and Characteristics | Reference |
|------|----|------|-------------------|-------------------|-----------------------------|------------|
| Gas1 | 60 | 559  |                   | N-terminal display | Required for cell wall assembly. | Ragni et al., 2007a |
| Gas3 | 57 | 524  | GPI anchor protein | N-terminal display | Involved in cell wall assembly and maintenance. | Ragni et al., 2007a |
| Gas5 | 52 | 484  | GPI anchor protein | N-terminal display | Required for the transfer of chitin to 1, 6-beta-glucan in the cell wall. | Cabib et al., 2007 |
| Mkc7 | 64 | 596  | GPI anchor protein | N-terminal display | Required for proper cell wall integrity and regeneration. | Gil-Bona et al., 2015 |
| Kre1 | 32 | 313  | GPI anchor protein | N-terminal display | Required for mating, normal hyphal development. | Samin et al., 2007 |
| Ycr89 | 166 | 1609 | GPI anchor protein | N-terminal display | Plays a role in maintenance of cell wall integrity during mating. | Castillejo et al., 2008 |
| Hwp1 | 65 | 634  | GPI anchor protein | N-terminal display | Required for cell wall integrity and structure. | Prill et al., 2005 |
| Crh1 | 53 | 507  | GPI anchor protein | N-terminal display | Required for the transfer of chitin to 1, 6-beta-glucan in the cell wall. | Cabib et al., 2007 |
| Crh2 | 50 | 467  | GPI anchor protein | N-terminal display | Involved in cell wall assembly and regeneration. | Cabib et al., 2007 |
| Crh11 | 47 | 453   | GPI anchor protein | N-terminal display | Required for the transfer of chitin to 1, 6-beta-glucan in the cell wall. | Cabib et al., 2007 |
| Crh12 | 57 | 504   | GPI anchor protein | N-terminal display | Required for proper cell wall integrity and structure. | Prill et al., 2005 |
| Ecm33 | 44 | 429   | GPI anchor protein | N-terminal display | Required for proper cell wall integrity and structure. | Prill et al., 2005 |
| Als1 | 133 | 1260 | GPI anchor protein | N-terminal display | Required for mating, normal hyphal development. | Samin et al., 2007 |
| Als3 | 124 | 1155 | GPI anchor protein | N-terminal display | Plays an important role in the pathogenesis of Candida albicans infections. | Prill et al., 2005 |
| Als5 | 142 | 1347 | GPI anchor protein | N-terminal display | Plays an important role in the pathogenesis of Candida albicans infections. | Prill et al., 2005 |
| Chr2 | 61 | 583   | GPI anchor protein | N-terminal display | Plays a role in cell separation. | Dünkler et al., 2005 |
| Exg2 | 63 | 562   | GPI anchor protein | N-terminal display | Exoglucanase. | Teparić and Mrša, 2013. |
| Pga4 | 49 | 451   | GPI anchor protein | N-terminal display | Involved in cell wall biosynthesis and morphogenesis. | Groot et al., 2003 |
| Pga5 | 72 | 641   | GPI anchor protein | N-terminal display | Involved in mating and cell wall assembly. | Groot et al., 2003 |
| Pga7 | 22 | 219   | GPI anchor protein | N-terminal display | Involved in heme-iron utilization. | Sogo et al., 2013 |
| Pga10 | 25 | 250   | GPI anchor protein | N-terminal display | Involved in heme-iron utilization. | Sogo et al., 2013 |
| Phr1 | 66 | 565   | GPI anchor protein | N-terminal display | Cell wall assembly and virulence. | Matsushika et al., 2016 |
| Phr2 | 59 | 544   | GPI anchor protein | N-terminal display | Cell wall assembly and virulence. | Matsushika et al., 2016 |
| Rhd3 | 21 | 204   | GPI anchor protein | N-terminal display | Component of the cell wall involved in virulence. | Castillo et al., 2008 |
| Sap9 | 58 | 544   | GPI anchor protein | N-terminal display | Required for cell surface integrity and cell separation during budding. | Albrecht et al., 2006 |
| Sap10 | 49 | 453   | GPI anchor protein | N-terminal display | Required for cell surface integrity and cell separation during budding. | Albrecht et al., 2006 |
2.2.2. Non-covalently linked cell wall proteins

This protein group comprises 9 members (Table 2), labeled as Scw1-Scw9; most of them are successfully purified and sequenced. They interact with the β-1,3-glucan network and forms hydrogen bond. These proteins are mainly O-glycosylated and contain a predicted N-terminal signal sequence. Actually these are non-covalently bounded proteins. SDS can be used to cleave this type of non-covalent interaction.

| Protein name | Molecular weight (KDa) | Length (a.a.) | Site of protein display | Functions (role in cell wall organization) | References |
|--------------|------------------------|---------------|------------------------|------------------------------------------|------------|
| Scw2         | 59                     | 562           | Not identified          | Endochitinase.                           | Hossain, 2018 |
| Scw3         | 43                     | 420           | Not identified          | Possibly involved in cell wall separation. | Teparić et al., 2010 |
| Scw4         | 40                     | 386           | Not identified          | Plays a role in conjugation during mating. | Grbavac, 2017 |
| Scw6         | 51                     | 448           | Not identified          | Exoglucanase.                            | Hossain, 2018 |
| Scw9         | 34                     | 313           | Not identified          | Endoglucanase.                           | Hossain, 2018 |
| Scw10        | 40                     | 389           | Not identified          | Plays a role in conjugation during mating. | Grbavac, 2017 |
| Scw11        | 56                     | 542           | Not identified          | Plays a role in conjugation during mating. | Grbavac, 2017 |
| Cts1         | 59                     | 562           | Not identified          | Chitinase/cell separation.               | Teparić and Mrša, 2013 |
2.3. Yeast cell wall proteins used in yeast surface display system

Table 1 and Table 2 showed that more than 80 proteins located on the surface of yeast cell. But in reality, all of them were not used as anchor proteins to construct yeast surface display system. In this work we tried to make an up to date list of anchored proteins used in construction of yeast display system till now (Table 3).

Table 3. Major cell wall anchor proteins with applications in yeast surface display systems.

| Anchor proteins | Applications (Target proteins to be fused on the yeast cell surface) | References |
|-----------------|---------------------------------------------------------------|------------|
| α-agglutinin    | Gluco-amylase, CM-cellulase, β-glucosidase, Endoglucanase, lipase B, EGFP, Celllobiohydrolase, Laccase, α-amylase | Shigechi et al., 2004; Qingjie et al., 2007; Inaba et al., 2009; Teparić et al., 2010; Yanase et al., 2010; Nakanishi et al., 2012 |
| Sed1            | β-Glucosidase, Endoglucanase, α-galactosidase                  | Teparić et al., 2010; Inokuma et al., 2016; Bamba et al., 2018 |
| Flo1p           | Glucoamylase, Streptavidin, Carboxylesterase (EstA), Organophosphorus hydrolase (OPH), α-amylase, Lipase B, R oryzae lipase, α-galactosidase | Shigechi et al., 2004; Furukawa et al., 2006; Breinig et al., 2006; Tanino et al., 2007; Teparić et al., 2010; Takeshi et al., 2010 |
| Ccw12           | RNase Rny1, Xylose reductase                                   | Teparić et al., 2013; Hossain et al., 2019 |
| Cwp1            | α-galactosidase, GFP                                          | Teparić et al., 2010; Xiaoyu et al., 2019 |
| Cwp2            | Lipase, Carboxylesterase (EstA), GFP, α-galactosidase          | Breinig et al., 2006; Teparić et al., 2010; Liu et al., 2010; Xiaoyu et al., 2019 |
| Tlr1            | α-galactosidase                                               | Xiaooy et al., 2019 |
| Tpl1            | α-galactosidase                                               | Teparić et al., 2010 |
| Ycr89           | α-galactosidase                                               | Teparić et al., 2010 |
| Pir1            | α-1,2-galactosyltransferase, α-1,2-mannosyltransferase        | Abe et al., 2003 |
| Pir2            | α-1,3-mannosyltransferase, α-2,3-sialyltransferase, α-1,3-fucosyltransferase VII | Abe et al., 2003; Salo et al., 2005 |
| Pir4            | Xylanase A, Lipase A                                          | Isabel et al., 2005; María et al., 2008 |

3. Discussion

In this study, we summarized 80 yeast cell wall anchor proteins. Several parameters are considered in order to recommend suitable yeast cell wall proteins for construction of more efficient yeast display system. For the simplification of this study, we categorized all this proteins into several classes based on they are they bonded to the cell wall; either covalently bonded or non-covalently bonded. Covalently bonded proteins constitute the major portion of yeast cell wall proteins. Two major classes of proteins are included in this category. One is the biggest protein group; the GPI-anchored cell wall proteins and another is PIR proteins group.

We found that a-agglutinin and α-agglutinin are two most widely used GPI-anchored cell wall proteins that helps to promote cellular aggregation during mating. A study revealed that this protein consist of 725 amino acids with high serine and threonine content, a putative N-terminal signal sequence, and a C-terminal hydrophobic sequence similar to signals for the attachment to GPI anchor (Hossain, 2018). While the other protein, α-agglutinin encoded by the AG alpha1 gene, has a similar function. Several surface display systems
with a-agglutinin and α-agglutinin developed by various researchers. Many construct have been designed to display and engineering various proteins as Gluco-amylase, CM-cellulase, β-glucosidase, Endoglucanase, lipase B etc. α-agglutinin is one of the most preferable anchor proteins to be used till now because of its several advantages.

Sed1 is another important cell wall anchor protein which has been used to display many heterologous proteins. This protein is involved in cell wall organization. It is a moderately weighted protein with 338 amino acids. The C-terminal domain of this protein is used as anchoring domain. Sed1 has been used to display several proteins like β-Glucosidase, Endoglucanase, α- galactosidase etc. Tir1, Tir2, Tir3, Tir4 proteins belong to the Tir protein family which have been used as an important anchor proteins. But Tir1 has been widely used because of its medium size and molecular weight. This protein has important roles in cell growth and survival. Some constructs have been made to display heterologous proteins on its cell surface like α-galactosidase. Tip1 is a medium sized GPI-anchored protein with 210 amino acids and plays important roles in lipase activity (Busch et al., 2004). This protein has been used before as anchor protein to display several heterologous proteins on cell surface. α-galactosidase, Human lactoferrin, GFP are some of the important examples (Xiaoyu et al., 2019).

Gas1 is a GPI-anchored cell wall protein with high molecular weight. This protein is required for spore wall assembly in Saccharomyces cerevisiae (Ragni et al., 2007a). Aldehyde dehydrogenase, β-galactosidase proteins were displayed by using Gas1 protein as anchor protein. Although, this protein has been used as cell surface anchor protein, it is better to avoid this protein because of its high molecular weight. Ycr89 is a high molecular weighted protein with long chain of about 1609 amino acids. This protein plays a key role in maintenance of cell wall integrity during mating. This anchor protein was used to display α-galactosidase on the cell surface (Xiaoyu et al., 2019). Ccw12 has been reported as the most abundant GPI-anchored cell wall protein that represents important structural component of the cell (Ragni et al., 2007b). Its main function is to maintain the newly synthesized areas of yeast cell wall. As well as this protein is required for the cell wall stability and integrity. Its molecular weight is about 13 KDa and composed of 133 amino acids. Ccw12 was successfully used in construction of surface display system for displaying several heterologous proteins. RNase Rny1, Xylose reductase etc are some of the good examples.

Cwp1 and Cwp2 are covalently linked cell wall proteins, which have been used to construct several yeast surface display systems. Its main function is to stabilize the cell wall. Cwp1 and Cwp2 both have been used to display several heterologous proteins like α-galactosidase, GFP and α-galactosidase, Carboxylesterase (EstA), GFP in their cell surface respectively. Sed1, Cwp2 and α-agglutinin were successfully used in construction of surface display system with varies degrees of success for displaying galactosidase (Xiaoyu et al., 2019) or GFP (Teparić et al., 2010) on the yeast surface. But an experiment showed that Cwp2 and Sed1 shows six to eight fold higher levels of displayed heterologous protein at the cell surface than that of α-agglutinin (Xiaoyu et al., 2019). Flo1 protein is one of the most used cell surface anchor proteins found in several yeast species. The Flo1 anchor system provides C-terminal or N-terminal fusion, but the high molecular weight of this anchoring domain showed the drawback in some cell wall display applications (Liu et al., 2010).

Another important class of covalently linked cell wall proteins are Pir proteins. The glucan chain is linked to the moderately weighted Pir protein composed of 341 amino acids could be a better choice as an anchor protein. But, the optimal fusion site of this protein has not been experimentally determined yet. But several constructs have been made to display foreign proteins on the cell surface (Abe et al., 2003). Pir2 is another important covalently linked cell surface anchor protein in which the optimal fusion site have not been experimentally determined. But several constructs have been made successfully because of its ideal features for displaying foreign proteins on cells surface. α-1,3-mannosyltransferase, α-1,3-fucosyltransferase, α-2,3-sialyltransferase, α-1,3-fucosyltransferase VII are some of the enzymes that have been displayed on the cells surface. Pir4 is the only member of Pir protein where the actual fusion site is experimentally determined. This medium sized protein composed of 227 amino acids. Study reported xylanase A as a displayed protein using Pir4 as the fusion partner (Andrés et al., 2005).

Seven of non-covalently linked protein group Scw1-Scw9 are successfully purified and sequenced (Hossain, 2018). Scw2 protein is possibly involved in cell separation after cytokinesis. Scw3 belongs to the group of SUN family proteins. Scw4 is another important member of this protein group with 386 amino acids. Possible role of this protein is in conjugation during mating. Bg12 is a major protein of S. cerevisiae cell wall with 34 KDa molecular size which shows lectin-like binding to β-1,3-glucan and chitin. This protein is involved in cell wall beta-glucan assembly (Teparić and Mrša, 2013). Scw10 is an important cell wall protein which is attached to the cell wall by non-covalent interaction. But this protein can be linked to the cell wall by alkali-sensitive linkage with a possible role in conjugation during mating. More researches are needed to clarify all the issues that could help future researchers in this field.
Experiment reported that both Cts1 and Cts2 plays important role in the cell separation process during growth (Teparić and Mrša, 2013). These are the anchoring proteins that have been used for more than two decades. But there are many yeast cell wall associated proteins that have not been used so far. Many of them plays important role in the stability as well as integrity of the cell wall. These proteins can be used as potential cell wall anchor proteins in the yeast surface display system.

We studied that two non-covalently cell wall attached proteins, Scw4 and Scw10 act as glucanases or transglucosidases in concert with other cell-wall proteins to assure cell-wall integrity (Grbavac, 2017). Another important cell wall anchor protein is Ccw12 that is considered as one of the most abundant yeast cell wall protein. It plays important role in maintenance of newly synthesized areas of cell wall and confers stability to the newly synthesized cell wall. Cwp1, Cwp2, Gas1, Exg1, Exg2, Ycr89. Crh1, Crh2 are considered potential GPI-anchored proteins responsible for the integrity and stability of the cell wall. Other proteins that confer stability to the cell wall; included all of the members of the Pir protein family, Cis1, Scw4, Scw6, Scw10, Exg1, and Bgl2.

4. Conclusions
For the construction of a successful yeast surface display system, one important prerequisite step is the identification and selection of a suitable cell wall anchor protein. The desired foreign protein, we want to display on the cell surface of yeast cell wall, must be attached to the anchor protein. Selection of an anchor protein is always a laborious task and needed to focus on several conditions. Several parameters should be determined and fixed prior to using the anchor protein in surface display system like molecular weight, fusion site, binding domain, catalytic domain are one of the important parameters.

We studied many cell wall proteins from several yeast species, focusing on these parameters. We categorized all these proteins into several classes based on their bonding nature. GPI-anchored proteins and Pir proteins belongs to the covalently bonded proteins group and non-covalently attached protein included Scw1-Scw10 proteins and others. Not all the proteins have been used to construct cell surface display system but some of them are extensively used than others and gain popularity. Most of them are covalently attached to the cell wall and medium weighted proteins. Our comparative study found that, these proteins can be used as best anchor proteins for the yeast surface display system and these are mainly α-agglutinin, Sed1, Cwp1, Ccw12, Tir1, Flo1, Ycr89, Gas1, Pir1, Pir2, Pir4, Exg2, Dan1. Our study found that, those cell wall proteins which are related to cell wall stability and integrity can be used as anchor proteins to construct yeast surface display system. These proteins have relatively good binding affinity to the cell wall and facilitate the targeted foreign protein’s identification and isolation process in later steps. Usually covalently linked proteins which are related to confer stability can get more advantages of it. But several non-covalently linked proteins like Scw4, Scw10 etc. proteins can also be used.

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Conflict of interest
None to declare.

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