Asian J. Med. Biol. Res. 2019, 5 (4), 246-257; doi: 10.3329/ajmbr.v5i4.45261

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Review

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

SK Amir Hossain*, SM Rifat Rahman, Toufiq Ahmed and Chanchal Mandal

Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh

*Corresponding author: SK Amir Hossain, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh. E-mail: isti_99@yahoo.com

Received: 14 November 2019/Accepted: 22 December 2019/ Published: 31 December 2019

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 bounded 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.

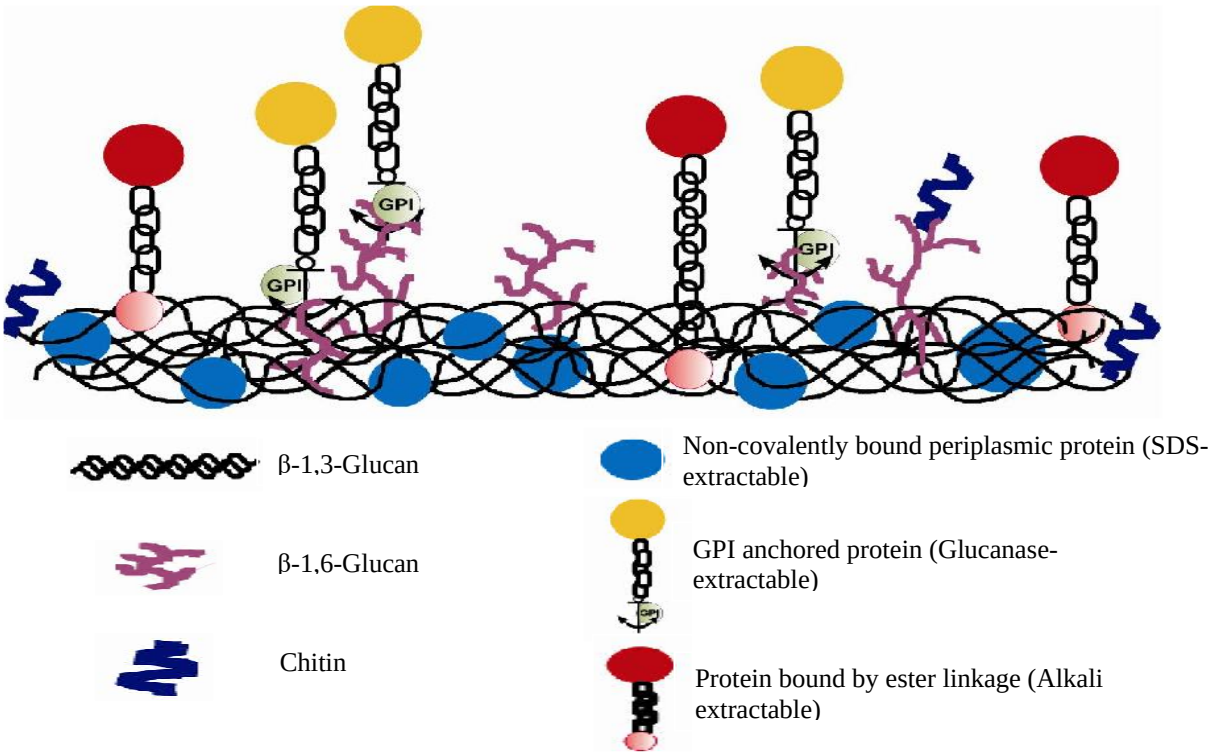

Figure 1. Architecture of yeast cell wall.

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, DGQφQ (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.

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

Gas1	60	559	GPI anchor protein	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	Involved in cell wall biosynthesis and morphogenesis.	Ragni *et al.*, 2007a
Mkc7	64	596	GPI anchor protein	N-terminal display	Required for cell wall integrity.	Krysan *et al.*, 2005
Kre1	32	313	GPI anchor protein	N-terminal display	Involved in cell wall 1, 6-beta-glucan synthesis and assembly.	Teparić and Mrša, 2013
Ycr89	166	1609	GPI anchor protein	N-terminal display	Plays a role in maintenance of cell wall integrity during mating.	Castillo *et al.*, 2008
Hwp1	65	634	GPI anchor protein	N-terminal display	Required for mating, normal hyphal development	Samin *et al.*, 2007
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	Required for the transfer of chitin to 1, 6-beta-glucan in the cell wall.	Cabib *et al.*, 2007
Crh11	47	453	GPI anchor protein	N-terminal display	Involved in cell wall assembly and regeneration.	Cabib *et al.*, 2007
Crh12	57	504	GPI anchor protein	N-terminal display	Involved in cell wall assembly and regeneration.	Cabib *et al.*, 2007
Ecm33	44	429	GPI anchor protein	N-terminal display	Required for proper cell wall integrity and structure.	Gil-Bona *et al.*, 2015
Als1	133	1260	GPI anchor protein	N-terminal display	Plays an important role in the pathogenesis of *Candida albicans* infections.	Prill *et al.*, 2005
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
Cht2	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 spore wall assembly.	Groot *et al.*, 2003
Pga7	22	219	GPI anchor protein	N-terminal display	Involved in heme-iron utilization.	Sorgo *et al.*, 2013
Pga10	25	250	GPI anchor protein	N-terminal display	Involved in heme-iron utilization.	Sorgo *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	N-terminal	Required for cell surface	Albrecht *et*

			protein	display	integrity and cell separation during budding.	*al.*, 2006
Ssr1	23	234	GPI anchor protein	N-terminal display	Involved in cell wall structure.	Yin *et al.*, 2005
Ywp1	54	533	GPI anchor protein	N-terminal display	Involved in cell adhesion.	Granger, 2012
Egt2	108	1041	GPI anchor protein	N-terminal display	Required for proper cell separation after cytokinesis.	Terashima *et al.*, 2003
Crr1	47	422	GPI anchor protein	N-terminal display	Chitosan-glucan cross-linking.	Teparić and Mrša, 2013
Pst1	46	444	GPI anchor protein	N-terminal display	Protect cell wall from oxidative stress.	Li *et al.*, 2015
Fig2	166	1609	GPI anchor protein	N-terminal display	Plays a role in maintenance of cell wall integrity during mating.	Zhang *et al.*, 2002
Kre9	30	276	GPI anchor protein	N-terminal display	Involved in cell wall 1,6-beta-glucan synthesis.	Teparić and Mrša, 2013
Pir1	35	341	Alkali sensitive ester linkage	C-terminal display	Required for cell wall stability.	Yang *et al.*, 2014
Pir2	41	413	Alkali sensitive ester linkage	C-terminal display	Required for cell wall stability.	Yang *et al.*, 2014
Pir3	33	325	Alkali sensitive ester linkage	C-terminal display	Required for cell wall stability.	Yang *et al.*, 2014
Pir4	23	227	Alkali sensitive ester linkage	C-terminal display	Required for cell wall stability.	Yang *et al.*, 2014
Hsp150	41	413	Alkali sensitive ester linkage	Not identified	Required for cell wall integrity, tolerant to heat shock.	Hsu *et al.*, 2015
Cis3	23	227	Alkali sensitive ester linkage	Not identified	Required for cell wall stability.	Yin *et al.*, 2005

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.

Table 2. List of non-covalently linked yeast cell wall proteins.

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

Cts2	59	511	Not identified	Chitinase/cell separation/sporulation.	Teparić and Mrša, 2013
Sim1	48	476	Not identified	Involved in cell separation.	Kuznetsov *et al.,* 2013
Mp65	39	378	Not identified	Required for cell wall integrity.	Sandini *et al.*, 2011
Uth1	37	365	Not identified	Involved in cell wall biogenesis.	Ritch *et al.,* 2010
Exg1	51	448	Not identified	Exoglucanase.	Teparić and Mrša, 2013
Bg12	34	313	Not identified	Endo- or trans-glucosidase.	Teparić and Mrša, 2013
Eng1	121	1117	Not identified	Endoglucanase/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, Cellobiohydrolase, 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
Tir1	α-galactosidase	Xiaoyu *et al.,* 2019
Tip1	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. Bgl2 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.

Acknowledgements

The authors are happy to thank the Biotechnology and Genetic Engineering Discipline, Khulna University for supporting this review work.

Conflict of interest

None to declare.

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