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Minireview

MINIREVIEW

Yeast-based vaccines: New perspective in vaccine development and application

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ABSTRACT

In presently licensed vaccines, killed or attenuated organisms act as a source of immunogens except for peptide-based vaccines. These conventional vaccines required a mass culture of associated or related organisms and long incubation periods. Special requirements during storage and transportation further adds to the cost of vaccine preparations. Availability of complete genome sequence, well-established genetic, inherent natural adjuvant and non-pathogenic nature of yeast species *viz. Saccharomyces cerevisiae*, *Pichia pastoris* makes them an ideal model system for the development of vaccines both for public health and for on-farm consumption. In this review, we compile the work in this emerging field during last two decades with major emphases on *S. cerevisiae* and *P. pastoris* which are routinely used worldwide for expression of heterologous proteins with therapeutic value against infectious diseases along with possible use in cancer therapy. We also pointed towards the developments in use of whole recombinant yeast, yeast surface display and virus-like particles as a novel strategy in the fight against infectious diseases and cancer along with other aspects including suitability of yeast in vaccines preparations, yeast cell wall component as an immune stimulator or modulator and present status of yeast-based vaccines in clinical trials.

Keywords: vaccine(s); yeast; yeast display; whole recombinant yeast; virus-like particles

INTRODUCTION

Despite the dramatic improvement in medical facilities, public health services and hygiene, infectious diseases remain a most important cause of human and farm animal sufferings across the globe and millions of people suffer and die every year from preventable infectious diseases. The emergence of antibiotic-resistant strains in pathogenic microbial population puts another hurdle in preventing human and animal suffering. Political instability and desire for high capital gain make the available vaccine out of reach those need them badly. Lack of necessary infrastructure for the proper supply and storage further increase the cost of conventional vaccines especially in developing countries of Asia and Africa.

Discovery of antibiotics in the early 1940s and their widespread application from 1960s onward have dramatically reduced the deaths and sufferings from microbial infections.

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Even then, infectious diseases are still the major threat to public health and farm animals, particularly in third-world countries. Blind applications of antibiotics in research, agriculture and public health led to the emergence of pathogenic strains that are no longer responsive towards available antibiotics (Davies 1994). In such scenarios, vaccine(s) (immunogen preparations that are deliberately injected into an individual for protection from infectious disease in future) remains the most important and safer way of combating infectious diseases. Therefore, there is an urgent need to look for new ways of vaccine generation that can cut down the cost, time and the need for special requirements during storage and distribution. In most of the conventional vaccine(s), dead/killed or attenuated/weakened organism(s) acts as a source of immunogens (Shams 2005). The arrival of DNA recombinant technology in the 1980s and parallel improvement in protein chemistry allows expression and purification of protein(s) in unlimited quantity, thus opening the door for peptide(s) or protein(s) vaccines as a prophylactic measure against infectious diseases, example peptide-based vaccine against Hepatitis B (Perrie *et al.* 2008).

CONCERNS RELATED TO CONVENTIONAL VACCINES

Although presently available vaccines proved to be highly valuable in the fight against infectious diseases and eradication of smallpox and soon polio is possible due to conventional vaccines. Despite these success stories, present vaccine preparation or application regime suffers from many drawbacks. For example, conventional vaccines require adjuvants and multiple injections for the induction of necessary or optimum immune response. Apart from that, these vaccine preparations pose another risk of pathogenicity with live attenuated vaccines reverting to virulence (Pastorest 1999; Shams 2005). The problem associated with the mass cultivation of pathogens of important infectious disease like leprosy, malaria defy present regime of vaccine development (Scollard *et al.* 2006; Cox 1991). The steady rise in the number of allergic or hypersensitivity response after vaccination in a certain group of individuals is another concern associated with conventional vaccines (Narasimhan *et al.* 2015; Fang *et al.* 2016). Administration of live or attenuated vaccine in the immunocompromised individuals (whose numbers is rising continuously due to new cases of HIV positive individuals and increase in the number of individuals with organ transplant who are on immunosuppressive drugs) is another point of concern (Rubin *et al.* 2014). Continuous maintenance of cold chain from manufacturing unit till endpoint user is another important issue associated with conventional vaccines (Das 2004; Chen and Kristensen 2009; Chen *et al.* 2011). Limitations of conventional vaccines are neatly summarised elsewhere (Pastoret 1999; Narasimhan *et al.* 2015; Fang *et al.* 2016; Rubin *et al.* 2014; Sun *et al.* 2016).

Thus, looking at the above points, there is an urgent need for novel vaccines with alternative routes of administration, low cost or suitability for resource-poor settings. In such conditions, scientists across the globe are busy in developing new and novel ways of vaccine preparation including DNA-based vaccines; expression of heterologous proteins in bacteria, plants, mammalian and insect's cells. Due to the reasons mentioned in the later section of the review, scientists focused their efforts on yeast as a suitable model system for the expression of heterologous proteins for vaccines development against infectious diseases and cancer .

In the present review, we compile a list of heterologous proteins expressed in different yeast species with the possibility of development of a yeast-based vaccines against infectious diseases along with cancer. We will also discuss novel ways of using yeast against infectious diseases including the use of whole recombinant yeast (WRY), yeast surface display (YSD) or simply yeast display (YD) and virus-like particles (VLPs) and proposed model which showed why whole recombinant yeast and yeast display is superior to the peptide or protein-based vaccines. How yeast cell wall components are important in modulating immune response is also discussed. All these aspects of yeast-based vaccines are described under separate heads.

SUITABILITY OF YEASTS FOR EXPRESSION OF THERAPEUTIC PROTEINS

Available tools of molecular biology and protein chemistry allow expression of proteins in almost any cells to be it prokaryotes (example *Escherichia coli*, *Bacillus subtilis*) or eukaryotes (example yeast, mammalian cells, cells of insects, in plants and animals). Despite this, yeast or unicellular fungus emerges as a model of choice for routine expression of proteins with medical importance. Some of the salient features that qualify yeast as a choice of model for routine expression of heterologous proteins for clinical or veterinary use are as follows. Unicellular fungus or yeast like *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Saccharomyces boulardii*, *Hansenula polymorpha, Pichia pastoris, Candida boldmu, Kluyveromyces lactis* and *Yarrowia lipolytica* are known to have highly efficient heterologous gene expression systems (Gellissen *et al.* 1992a; Gellissen *et al.* 1992b; Gellissen and Hollenberg 1997; Walker 1998; van Ooyen *et al.* 2006). Apart from the yeast species mentioned earlier, *Blastobotrys* (*Arxula*) *adeninivorans* also emerges as an important host for expression of heterologous proteins due to various benefits associated with this yeast species including its ability to use a wide range of substrate, availability of genome sequence, responsive to genetic manipulation (Malak, Baronian and Kunze 2016). Comparative advantages and disadvantages of different yeast species as a host for expression of heterologous proteins are discussed elsewhere (Vieira Gomes *et al.* 2018). Out of hundreds of yeast species, *S. cerevisiae* and *P. pastoris* emerges as a most common host for the expression of heterologous genes and the production of therapeutic proteins (Darby *et al.* 2012). So far *S. cerevisiae* is the most well-understood eukaryote at the molecular level and is commonly used yeast in brewing and baking for many years. It has been used for several biotechnological purposes due to its cheap and easy cultivation, well-established fermentation processes and large-scale production capabilities (Gellissen and Hollenberg 1997; Walker 1998). Information on its genetics, molecular biology and physiology has been accumulated, ensuring that this organism is a highly available eukaryotic system (Strathern, Jones and Broach 1982; Rose and Harrison 1987; Broach, Pringle and Jones 1991). Several selective promoter elements and mutations in this yeast species was investigated for an increased yield or an improved quality of recombinant products (Gellissen and Hollenberg 1997). Some of the cell components of yeast, act as natural adjuvants with the ability to stimulate or modulating immune response, which is important in using WRY or yeast display for oral application. Due to its simple growing method and non-toxic nature, it is reasonable to believe that such recombinant yeast can be further developed into an edible vaccines. Many heterologous proteins, including the first commercialised recombinant vaccine, the Hepatitis B vaccine, were produced

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in *S. cerevisiae,* in which genetic engineering techniques were applied (Valenzuela *et al.* 1982; Gellissen and Hollenberg 1997). It is also known that living recombinant budding yeast secretes proteins or peptides in mice gut, which opens the way for the development of a live oral vaccine (Blanquet *et al.* 2004).

Although many heterologous proteins have been successfully expressed in *S. cerevisiae*, its limitations mean that alternative, non-*Saccharomyces* yeasts have to be examined (Gellissen and Hollenberg 1997; Cregg *et al.* 1987; Gellissen *et al.* 1992a). Generally, heterologous proteins expressed in *S. cerevisiae* have low yields and are hyperglycosylated, which may result in differences in immunogenicity, diminished activity or decreased serum retention of the foreign protein (Cregg *et al.* 1987). *Pichia pastoris* is a methylotrophic yeast that uses methanol as its sole energy and carbon source (Gellissen and Hollenberg 1997). The genes encoding key enzymes of the methanol-utilisation pathway, which are generally used by the methylotrophic yeasts, provide inducible promoters for the efficient expression of heterologous DNA sequences (Gellissen and Hollenberg 1997). In addition, *P. pastoris* produces correctly folded and secreted proteins into the medium and is capable of performing post-translational modifications that are closer to human protein modifications than those produced by *S. cerevisiae* (Cino 1999). Since *P. pastoris* can grow on a simple mineral media and secretes low levels of endogenous proteins, the heterologous protein comprises the major portion of the total protein in the medium thus simplifying the purification process (Lin *et al.* 2009).

K. lactis or milk yeast is one of the few yeast species that can grow on lactose and whey; it is cheap and uses various substrates, as a sole source of carbon and energy (Romanos, Scorer and Clare 1992; Arnold *et al.* 2012). Its potential as a host for the production of heterologous proteins has been studied especially for low-value products (Romanos, Scorer and Clare 1992). For example, *K. lactis* can efficiently synthesise and secrete fully active foreign proteins, including prochymosin, which is poorly secreted by *S. cerevisiae* (ven Dan Berg *et al.* 1990; Romanos, Scorer and Clare 1992). In addition, this yeast allows rich biomass yields during fermentation at high growth rates (Arnold *et al.* 2012).

Availability of molecular tools allowed the expression of heterologous proteins and their display on the cell surface (Schreuder *et al.* 1993; Schreuder *et al.* 1996; Ueda and Tanaka 2000). Numerous antigens from different pathogens including Hepatitis B virus, porcine epidemic diarrhoea virus, *Actinobacillus pleuropneumoniae*, protozoans were successfully expressed on yeast surface for the development of oral vaccines (Schreuder *et al.* 1996; Park *et al.* 2007; Kim *et al.* 2010a). Thus, unicellular yeast can be the right candidate for a live oral vaccine (Schreuder *et al.* 1996; Ueda and Tanaka 2000). Figure 1A shows the different group of pathogens against which immunogenic protein(s) was expressed in yeast, while Fig. 1B shows the ways in which yeast can be used for vaccines development.

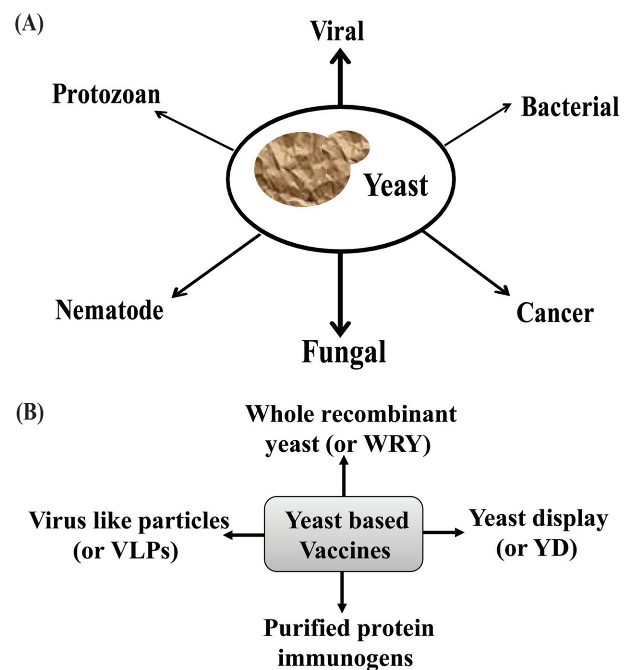

Figure 1. Yeast-based vaccines. (A) Schematic showing the application of yeast in an expression of antigens against diverse clinical conditions. (B) Various ways in which yeast-based vaccines are or can be used.

YEAST AND IMMUNE RESPONSE

Despite its non-pathogenic nature (*S. cerevisiae, P. pastoris*), yeast has been shown to induce immunologic responses in mammals and is avidly taken up by DCs (dendritic cells) and macrophages (Stubbs *et al.* 2001; Heintel *et al.* 2003). Immunogenic nature of yeast cells was believed due to polysaccharides beta-1, 3-D-glucan (BGs), and mannan that allows efficient phagocytosis of yeast cells by APCs (antigen-presenting cells) including DCs, followed by generation of danger signals during microbial infection. These carbohydrates moieties possess strong adjuvant nature that is easily detected by pattern recognition receptors like toll-like receptors (TLRs) and mannan receptors on APCs (Munson *et al.* 2008).

Yeast cells expressing foreign antigens is degraded in proteasomes and latter displayed on the cell surface through MHC-I, which is recognised by CD81 (cluster of differentiation) CTLs (cytotoxic T lymphocytes) that leads in proliferation, maturation and activation of antigen-specific CD81 CTLs. In another case, yeast cells can also be degraded in endosomes, and antigenic peptides are again presented on the cell surface through MHC-II, which are recognised by CD41 T helper cells that are important in T-cell immunity. Apart from that yeast cells are also known for their ability to enhance expression of MHC (major histocompatibility complex) and co-stimulatory molecules on a surface of DCs or APCs (Stubbs *et al.* 2001; Franzusoff *et al.* 2005; Bernstein *et al.* 2008; Munson *et al.* 2008; Remondo *et al.* 2009) thereby allowing efficient activation of T lymphocytes for cell-mediated immunity.

It is also evident that yeast cells (budding yeast and Candida) are also able to increase the interleukin (IL) secretion including IL 6, 8, 12, 18, TNF, MCP-1/CCL2 along with IL-1beta. The same study also showed an increased expression of TLR including TLR1, 2, 4, 6 along with dectin (Saegusa *et al.* 2009). Application of Zymosan (from yeast cells) to mast cells increased dectin-1 expression and also leads to induction of dectin-1-dependent reactive oxygen species (ROS) generation (Yang and Marshall 2009) while yeast glycoprotein was able to induce proliferation of human lymphocytes (Darroch, Christmas and Barnes 1994). Applications of VLPs derived from yeast were able to activate HIV-1 Gag-specific $CD8^{+}$ T cells (Mizukoshi *et al.* 2009). The inclusion of yeast cell wall extract in the feed of pig was able to boost innate and acquired immunity and growth (Sauerwein, Schmitz and Hiss 2007).

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Recently it is also reported that M1-like macrophages had a higher yeast uptake capacity compared to M2-like macrophages and both types of macrophages were equally efficient in their ability to take opsonised yeast cells. Apart from that, uptake of yeast by macrophages also leads to an increase in surface markers (example HLAII and CD86). Moreover, mRNA levels of pro-inflammatory cytokines, such as TNF-α, IL-12, and IL-6, increased, while that of anti-inflammatory mediator's remains changed (Seif *et al.* 2016). It is also shown that whole recombinant budding yeast expressing foreign antigens can confer protective cell-mediated immunity against a tumour (Stubbs *et al.* 2001). It is important to mention that yeast-based vaccines were able to generate antigen-specific responses independent of the viability of the cells itself (Lu *et al.* 2004) and magnitude of immune response were of the same level on an application of either dead or alive cells (Franzusoff *et al.* 2005). Components of yeast cell wall were able to interact with DCs cell and macrophage receptors (TLR-2, TLR-4, TLR-6, CD14, Dectin-1, Dectin-2, DEC-205 and the mannose receptor family) and are highly efficient in stimulating antigen presentation (Gantner *et al.* 2003; Underhill and Gantner 2003). Zymosan from yeast cell wall was able to increase the expression of many pro-inflammatory genes (Tada *et al.* 2002; Underhill and Gantner 2003; Ikeda *et al.* 2005). Thus, from looking at these studies, it can be said that yeast cell is able to mount and modulate the immune response, further showing the significance of inherent adjuvant nature of yeast.

OPTIMISATION OF TRANSCRIPTION AND TRANSLATIONAL APPARATUS

Quick and cost-effectiveness are two important elements associated with the use of yeast for the expression of heterologous proteins for clinical applications. To achieve these endpoint objectives, it is important that the target gene(s) expressed in yeast remain stable. Although several vector systems are available for efficient expression of heterologous proteins in yeast. Under such a condition, it is important that one must find the most suitable and economically viable strategy for protein expression. The strategy employed in protein expression should also allow rapid and economical protein purification without affecting native protein property especially its immunogenic nature. In another case, protein purification may not be required (example in whole recombinant yeast and yeast display as discussed in the later section of the review) and increased expression and display of protein on yeast surface (without compromising on immunogenic property) may be important. Thus, it is important to consider the endpoint application before deciding the strategy for expression of heterologous proteins and accordingly selects the expression system (to be it host i.e. yeast species as well as expression vectors).

Although one can use episomal-based expression vector, these expression vectors need continuous selection pressure for maintaining the clones. Selection through the antibiotic does not sound good in case of whole recombinant yeast and yeast display. Auxotrophic selection can help in getting rid of antibiotics but poor and slow growth in these growth media is another issue. Although the application of integrating the vector allows long-term maintenance of the clones on rich media even in the absence of selection pressure, the number of copies of antigen that can be integrated through integrating vector is another issue

Thus, for efficient and economical expression of heterologous protein in yeast, a novel strategy that can surmount issue mentioned earlier is a must. Efforts over the years have led to the development of various strategies as pointed here. The detailed strategy is beyond the scope of present review and readers are advised to check original papers that describe the experimental strategy in sufficient detail.

For permanent, long-term maintenance and expression of a clone(s) of interest, a gene can be integrated into non-transcriptional spaces (NTSs) of yeast rRNA gene as chromosomal integration sites. One can also integrate a clone of interest at yeast chromosomal centromere or telomere regions (Hohmann 1987). To overcome the problem of copy number non-transcribed spacers, NTS1 and NTS2, in the 5' and 3' end flanking regions of 5S ribosomal DNA can be used (Wery *et al.* 1997; Klabunde *et al.* 2002). For example, by using this strategy high copy number of mammalian myostatin was integrated into rDNA region of budding yeast and antibiotic selection marker was removed using the Cre-LoxP system to rule out the side effects that may arise due to the presence of an antibiotic gene. This allows maintenance of yeast in nutrient-rich media for rapid growth and expression of mammalian myostatin for two years. Further oral application of these yeast cells was able to induce an immune response (Zhang *et al.* 2012). Similarly, new, multiple integration cassettes were developed by exploiting the NTS of rDNA in combination with defective selection markers. In this case, 5' and 3'-fragments of rDNA-NTS2 were used for expression cassettes with a set of *URA3, LEU2, HIS3* and *TRP1* selection markers with truncated promoters of different lengths. The number of integrated cassettes may range up to 30 copies. This approach was tested by constructing yeast strain capable of expressing capsid protein of red-spotted grouper necrosis virus (RG-NNVCP). The oral administration of this recombinant yeast was able to mount efficient immune responses (Moon *et al.* 2016). In another recent study, using *P. pastoris* expression strategy was devised that allows efficient expression and easy purification of HPV16 L1 protein by inserting codon optimised α-factor signal sequence (Mariz *et al.* 2015). Thus, it can be said that available tools and improvement over the years allows efficient expression of heterologous proteins without compromising the ability of expressed proteins to interact with immune cells or molecules and mount an immune response.

EXAMPLE OF PROTEINS EXPRESSED IN YEAST AGAINST A DIFFERENT GROUP OF PATHOGENS

Viral proteins

The high rate of mutation, the absence of DNA repair systems and requirement of a host or cellular environment put serious challenges in the development of vaccines against viral infections. Appearance or emergence of newer and lethal viral strains now and then or appearance of new pathogenic viruses (example H1NI, H1N5 and more recently Ebola and Zika virus) poses another hurdle in vaccine development against viral infections. Due to the reasons mentioned earlier, vaccines against even common viral infection like Hepatitis C remains a challenge. But the better understanding of the viral genome, molecular techniques, and protein chemistry allow mass production of important viral proteins for use as prophylactic vaccines. For example, the introduction of several copies of cassettes encoding the major Hepatitis B surface protein into *S. cerevisiae* proved

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useful for vaccine applications (Jacobs *et al.* 1989). DENV envelope (E) protein-based VLPs generated by using *P. pastoris* shows encouraging results against dengue (Mani *et al.* 2013) and purification of gp120 of HIV-1 from *P. pastoris* shows positive response against HIV (Scorer *et al.* 1993). *H. polymorpha* allows high-level expression and purification of gp96 of HBV, which can effectively induce HBV-specific CTL response in immunised mice (Li *et al.* 2011). Heat-killed whole recombinant budding yeast-based vaccine (GS-4774) against Hepatitis B virus reached to phase-II of clinical trials (Lok *et al.* 2016). As a proof of concept, we mentioned some of the studies where expression of viral proteins in yeasts was able to induce an immune response against viral infection, and the list of various viral proteins expressed in different yeasts species are shown in Table 1.

Protozoan proteins

The causative agents of human malaria are four species of protozoan parasites belonging to the genus *Plasmodium*. Although *P. falciparum* is responsible for the highest mortality rate among the four species of human malaria parasites, *vivax* malaria is responsible for the most recurrent form of malaria (Mendis *et al.* 2001). Antibodies raised by using Pvs25 and Pvs28 expressed and purified from yeast were able to block transmission of *P. vivax* in mice (Duffy, Pimenta and Kaslow 1993). Chimeric protein 2.9 (PfCP-2.9) secreted by *P. pastoris* was able to induce the generation of antibodies and inhibit the growth of *P. falciparum CC1/HN* and 3D7 lines in rabbit and rhesus monkeys (Pan *et al.* 2004). Table 2 shows the list of protozoan spp. proteins expressed in different yeast as a potential vaccine against malaria. Compared to budding yeast, P30P2MSP1(19) secreted by *P. pastoris* generate high and uniform antibodies titer in rabbit (Brady *et al.* 2001). Thus, the problem of the mass culture of *Plasmodium* species for vaccine development may be overcome by expressing surface proteins (from different stages) in yeast, and it is expected that in near future, yeast expressing *Plasmodium* protein(s) may occupy an important place in malaria treatment.

Bacterial proteins

Due to the availability of different antibiotics and ability, to culture bacteria of almost all pathogenic species (except members of *Mycobacteria* genus) research in the use of yeast for vaccine development against bacterial infection(s) is limited. As a result, compared to a virus or protozoa few reports are available where bacterial proteins were expressed in yeast for vaccines development. For example, administration of mycobacterial heparin-binding haemagglutinin adhesin (HBHA) expressed and purified from *P. Pastoris* was able to induce Th1-type immunity and reduced bacterial load in mice spleen (Kohama *et al.* 2008). Oral application of whole recombinant budding yeast expressing ApxIIA provides protection against *A. pleuropneumoniae* (Shin *et al.* 2007, 2013). The list of bacterial protein expressed in yeast for use as vaccines are listed in Table 3.

Fungal proteins

Although the incidence of fungal infection is less compared to bacterial or viral infection, the eukaryotic nature of fungal cells poses an important challenge, and thus, the development of therapeutic measure against fungal infection is slow. For example, only limited compounds are available for treatment of fungal infection and development of a vaccine against fungal infection remains a challenge. And as a result, scientists focused their attention on cell wall component as a potential target for a medical intervene. Conserved nature of cell wall components particularly polysaccharides like beta glucan, mannans, zymogen and their absence in the host including mammals, poultry birds make them a suitable candidate for inducing the immune response against fungal infection. The use of a vaccine preparation using hemolysin expressed in *S. cerevisiae* vector has been reported and observed that the empty vector also conferred partial protection in an infection model of coccidioidomycosis (Capilla *et al.* 2009). Administration of heat-killed yeast (HKY) provides effective protection against aspergillosis and coccidioidomycosis (Stevens, Clemons and Liu 2011), coccidioidomycosis (Capilla *et al.* 2009), candidiasis. Injection of budding yeast expressing *P. brasiliensis* gp43 was able to protect mice from paracoccidioidomycosis (PCM) (Assis-Marques *et al.* 2015) while application of plasma bead coated with *C. albicans* cytoplasmic proteins provides a positive immune response against candida infection (Ahmad *et al.* 2012). Thus, it can be said that vaccine against fungal infection is still a wish and yeast-based vaccine may emerge as an important way in therapy against fungal infection.

Nematodes and ticks proteins

In this section, we highlighted some of the studies where yeast has been used for expression of proteins from nematodes or worms and ticks or arthropods from the point of therapeutic measures. For example, tetraspanin surface antigen protein (Sm-TPS2) expressed and purified from yeast showed positive response against *Schistosoma mansoni* in mice (Cheng *et al.* 2013). Administration of glutathione S-transferase 1 (Na-GST-1) purified from yeast shows encouraging results against *Necator americanus* infection (Zhan *et al.* 2010; Plieskatt *et al.* 2012; Curti *et al.* 2014) while aspartyl protease inhibitor (API-1) purified from yeast showed a protective immune response against *Ancylostoma caninum* and *Ancylostoma ceylanicum* (Delaney *et al.* 2005). Application of proteins expressed and purified from yeast shows protective immunity against *Boophilus microplus* (García-García *et al.* 1998, 2000). Application of proteins expressed in yeast showed positive response against *Ascaris suum* (Wei *et al.* 2017). Similarly, *S. mansoni* Sm14 antigen expressed in *P. pastoris* showed positive response in preliminary clinical trials (Damasceno, Ritter and Batt 2017). Different nematode and ticks' proteins expressed in yeast are shown in Table 4.

CANCER-ASSOCIATED PROTEINS

For any successful or positive response against a tumour, it is important that the yeast-based vaccine should be able to generate the CD81 CTLs cells important in both recognition and killing of tumour cell(s). Therefore, yeast cells capable of expressing a proper and optimum level of tumour-specific or tumour-associated antigens need to be constructed. Numerous clinical studies with this strategy are under way and reviewed elsewhere (Ardiani, Higgins and Hodge 2010). In one preclinical study using *S. cerevisiae* expressing brachyury (embryonic transcription factor) was able to activate human T cells (Heery *et al.* 2015). *S. cerevisiae*-derived microparticles conjugated to ova albumin was recognised and internalised by DCs and shown to induce an immune response against a tumour (Pan *et al.* 2015). Another study reported the phase I trial of a recombinant *S. cerevisiae* -CEA vaccine (GI-6207) in adults with metastatic CEA-expressing carcinoma (Bilusic *et al.* 2014) and NY-ESO-1

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Table 1. Table showing list of various viral proteins expressed in various yeast species.

Yeast species	Source of antigen/Diseases	Antigen/Immunogen	Expression strategy	Reference
S. cerevisiae	Papillomavirus/Human Papilloma	HPV16 L1 protein	*Virus like particle	Kim *et al.* 2007
		VP1	Virus like particle	Zielonka *et al.* 2012
		HPV-11 L1	Virus like particle	Brown *et al.* 2001
		Papillomavirus type 16 L1 protein	Purified protein	Park *et al.* 2008
		Type 16 L1 protein	Virus like particle	Kim *et al.* 2010b
		HPV16	Virus like particle	Kim *et al.* 2014
		HPV16 L1	Virus like particle	Woo *et al.* 2007
		HPV18 L1	Virus like particle	Woo *et al.* 2008
		VP1	Virus like particle	Sasnauskas *et al.* 2002
		HPV16 E7 oncoprotein epitopes	Virus like particle	Pumpens *et al.* 2002
		Capsid proteins L1 or L1 plus L2	Virus like particle	Lowe *et al.* 1997
		HPV58 L1	Virus like particle	Kwag *et al.* 2012
	Hepatitis B virus/Hepatitis	HBsAg	Virus like particle	Kee *et al.* 2008
		Hepatitis B surface antigen	Purified protein	Jacobs *et al.* 1989
		Hepatitis B surface antigen (HBsAg)	Virus like particle	Hadiji-Abbes *et al.* 2013
		Hepatitis B virus (HBV) X, surface (S), and Core antigens (X-S-Core)	Whole recombinant yeast	King *et al.* 2014
		Surface protein GS-4774	Whole recombinant yeast	Gaggar *et al.* 2014
		HBsAg	Virus like particle	Pleckaityte *et al.* 2015
		Hepatitis B core protein	Purified protein	Zhang *et al.* 2015a
		Hepatitis B surface antigen	Purified protein	Hauser *et al.* 1987
		Hepatitis B surface antigen	Purified protein	Tan *et al.* 1994
	Hepatitis C virus/Hepatitis	HCV NS3-Core fusion protein	Whole recombinant yeast	Haller *et al.* 2007
	Enterovirus 71/Foot, hand and mouth disease	EV71 structural protein	Virus like particle	Li *et al.* 2013
		EV71 structural proteins VP1-VP	Virus like particle	Wang *et al.* 2016a
		VP1	Virus like particle	Lin *et al.* 2015
		ChiEV-A71 VLPs	Virus like particle	Zhao *et al.* 2015
	Coxsackievirus A16/Foot, hand and mouth disease	ChiEV-A71 VLPs	Virus like particle	Zhao *et al.* 2015
		P1 and 3CD	Virus like particle	Zhao *et al.* 2013
	Japanese encephalitis virus/Encephalitis	Japanese encephalitis virus envelope protein (mannoprotein)	Yeast surface display	Upadhyaya and Manjunath 2009
	Puumala hantavirus (PUUV)/Hemorrhagic Fever	Nucleocapsid protein	Purified protein	Dargeviciute *et al.* 2002
	Hamster polyomavirus/Epithelioma (Syrian hamsters)	Hamster polyomavirus (VP1)	Virus like particle	Gedvilaite *et al.* 2000
		Capsid protein VP1	Virus like particle	Mazeike *et al.* 2012
	Parvovirus/Aplastic crisis		Virus like particle	Penkert *et al.* 2017
	Parvovirus B19/Aplastic crisis	VP1 and VP2	Virus like particle	Chandramouli *et al.* 2013
	Dengue virus/Dengue	Dengue envelope domain III (scEDIII)	Purified protein	Nguyen *et al.* 2013
	HIV-1/AIDS	HIV-1 envelope (Env) glycoprotein	Yeast surface display	Wang *et al.* 2018a
		Retroviral Gag protein	Virus like particle	Sakuragi *et al.* 2002
		Gag protein	Virus like particle	Tomo *et al.* 2013

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Table 1. Continued

Yeast species	Source of antigen/Diseases	Antigen/Immunogen	Expression strategy	Reference
	Red-spotted grouper NNV (RGNNV)/Nervous necrosis	Capsid protein	Whole recombinant yeast (oral application)	Cho *et al.* 2017
	Simian-human immunodeficiency chimeric viruses/AIDS	A pool of SHIVenv_B derived from 16 acute HIV-1 infections	Chimeric virus	Krebs *et al.* 2016
	Varicella-zoster virus/Chickenpox	Varicella-zoster virus (VZV) gE	Virus like particle	Welsh *et al.* 1999
	Nervous necrosis virus (NNV)/Nervous necrosis	RGNNV	Virus like particle	Wi *et al.* 2015
		RGNNV capsid protein	Virus like particle	Choi *et al.* 2013
	Rotavirus/Gastroenteritis	Rotavirus VP2, VP6 and VP7	Virus like particle	Rodri´guez- Limas *et al.* 2011
	Goose hemorrhagic polyomavirus/Hemorrhagic nephritis and enteritis of geese (HNEG)	VP1	Virus like particle	Zielonka *et al.* 2006
	Cottontail rabbit papillomavirus (CRPV)/Cancer	Capsid proteins L1 or L1 + L2	Virus like particle	Jansen *et al.* 1995
	New castle virus/Mild flu-like symptoms or conjunctivitis (an infection of the eye that is also called pink eye) and/or laryngitis	Hemagglutinin-neuraminidase	Purified protein	Khulape *et al.* 2015
P. pastoris	Dengue virus/Dengue	DENV envelope protein domain III (EDIII)	Virus like particle	Ramasamy *et al.* 2018
		Dengue Virus 4 Envelope Glycoprotein	Virus like particle	Khetarpal *et al.* 2017
		DENV-2 and DENV-3 envelope (E) glycoproteins,	Virus like particle	Poddar *et al.* 2016
		DENV1–4	Virus like particle	Liu *et al.* 2014
		DENV-2 E	Virus like particle	Mani *et al.* 2013
		Envelope Glycoprotein E	Virus like particle	Liu *et al.* 2010
		DENV-1	Virus like particle	Tang *et al.* 2012
		Dengue virus type-2 (DENV-2) envelope domain III (EDIII)	Virus like particle	Arora *et al.* 2013
		Envelope domain III protein	Purified protein	Etemad *et al.* 2008
		Envelope protein (E1-E4)	Virus like particle	Rajpoot *et al.* 2018
	Rock bream iridovirus (RBIV)/Morbidity and mortality in fish	Recombinant major capsid protein (rMCP)	Whole recombinant yeast	Seo *et al.* 2013
	M. tuberculosis/Hepatitis	Tbhsp70-HBcAg (18–27)	Purified protein	Peng *et al.* 2006
	Dengue virus type 2 and Hepatitis B virus/Dengue, Hepatitis B	Den2E-HBsAg	Virus like particle	Bisht *et al.* 2002
	Coxsackievirus A/Hand, foot and mouth disease (HFMD)	P1 and 3CD	Virus like particle	Zhou *et al.* 2016
		P1 and 3CD proteins of CA16	Virus like particle	Zhang *et al.* 2016
	Enterovirus/Hand, foot and mouth disease (HFMD)	P1 and 3CD proteins of EV71	Virus like particle	Zhang *et al.* 2015b
		P1 precursor and 3CD protease	Virus like particle	Zhang *et al.* 2018

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Table 1. Continued

Yeast species	Source of antigen/Diseases	Antigen/Immunogen	Expression strategy	Reference
	Hepatitis B virus/Hepatitis	HBsAg and HEnAg fusion protein	Virus like particle	Li *et al.* 2004
		HBsAg	Virus like particle	Zahid *et al.* 2015
		Core protein (HBc)	Virus like particle	Freivalds *et al.* 2011
		Recombinant hepatitis B surface antigen	Purified protein	Hardy *et al.* 2000
		HBsAg, HSP70 (1–370)	Whole recombinant yeast	Zeng *et al.* 2003
		HBsAg	Virus like particle	Gurramkonda *et al.* 2009
		Hepatitis B surface antigen	Virus like particle	Lu¨nsdorf *et al.* 2011
		Recombinant hepatitis B surface antigen	Virus like particle	Liu *et al.* 2008
	Hepatitis C virus/Hepatitis	HCV CoreE1E2 protein	Purified protein	Fazlalipour *et al.* 2015
		Hepatitis C core protein (HCcAg)	Virus like particle	Acosta-Rivero *et al.* 2001
	Papillomavirus type 16/Papilloma/cancer	HPV16L1/18L1	Virus like particle	Hanumantha Rao *et al.* 2011
			Virus like particle	Jiang *et al.* 2011
		HPV 16 and 18 L1	Virus like particle	Gupta *et al.* 2017
		HPV-16 L1	Purified protein	Coimbra *et al.* 2011
		Papilloma virus type 16 (HPV-16) L1-L2 proteins	Purified protein	Bredell *et al.* 2018
	Infectious bursal disease virus/Immunosuppression	IBD-VP2	Virus like particle	Wang *et al.* 2016b
	Giant salamander iridovirus/Mortility in Chinese giant salamander	O-MCP	Virus like particle	Zhou *et al.* 2015
	Duck Hepatitis 1/Hepatitis (in duck)	VP1 protein	Purified protein	Wang *et al.* 2014
	Influenza virus/Flu	Recombinant Hemagglutinin protein of novel H1N1	Purified protein	Athmaram *et al.* 2011
		Hemagglutinin protein	Purified protein	Athmaram *et al.* 2013
		H5 haemagglutinin	Purified protein	Pietrzak *et al.* 2016
		Full-length HA-encoding gene of H5N2 AIV	Purified protein	Wang *et al.* 2007
		Alpha aggulutinin	Yeast surface display	Wasilenko *et al.* 2010
		Hemagglutinin protein	Purified protein	Wang *et al.* 2018b
	Porcine circovirus/Postweaning multisystemic wasting syndrome (PMWS)	Porcine circovirus type 2 capsid	Purified protein	Tu *et al.* 2013
	HIV/AIDs	HIV envelope protein gp120	Purified protein	Scorer *et al.* 1993
	Bovine herpesvirus-1/Rhinotracheitis, vaginitis, balanoposthitis, abortion, conjunctivitis, and enteritis	Bovine herpesvirus type 1 (BHV-1) glycoprotein D (gD)	Purified protein	Zhu *et al.* 1999
		Bovine herpesvirus-1 (BHV-1) glycoprotein D (gD)	Purified protein	Zhu *et al.* 1997
	Classical swine fever virus (CSFV)/Swine fever (CSF) or hog cholera	Glycoprotein E2	Purified protein	Cheng *et al.* 2014
	Avian influenza virus/Influenza	Influenza A/H5N1 neuraminidase	Purified protein	Yongkiettrakul *et al.* 2009
	Bursal disease virus (IBDV)/Bursal disease	VP2 gene of the Edgar strain of IBDV, hypervariable region of the VP2 gene (hvVP2)	Purified protein	Villegas *et al.* 2008

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Table 1. Continued

Yeast species	Source of antigen/Diseases	Antigen/Immunogen	Expression strategy	Reference
	Avian leukosis virus/Cancer in chickens	Recombinant J subgroup of avian leukosis virus (ALV-J) gp85 protein	Purified protein	Jing *et al.* 2018
	H. pylori/Gastritis, peptic ulcer disease and gastric cancer	Alkyl hydroperoxide reductase (AhpC)	Purified protein	O'Riordan *et al.* 2012
H. polymorpha	Human Papillomavirus/Papilloma/cancer	HPV52L1	Virus like particle	Liu *et al.* 2015
	Hepatitis B/Hepatitis	VrHB-IB	Purified protein	Caetano *et al.* 2017
	Foot-and-mouth disease virus/Foot-and-mouth disease	Capsid protein complex epitopes of VP1, cholera toxin B subunit	Purified protein	Song *et al.* 2004
	Hepatitis C virus/Hepatitis	Envelope glycoprotein E1	Virus like particle	He *et al.* 2009
		Envelope glycoprotein E1 ectodomain	Purified protein	Lorent *et al.* 2008
	Bovine viral diarrhea virus (BVDV), Classical swine fever virus (CSFV), Feline leukemia virus (FeLV), West nile virus (WNV)/Infectious disease in animals	Membrane integral small surface protein (dS) of the duck hepatitis B virus fused with virus antigen infecting animals	Virus like particle	Wetzel *et al.* 2018
	Porcine circovirus type 2b (PCV2b)/Postweaning multisystemic wasting disease (PMWS) in pigs	PCV2b capsid protein (CP)	Virus like particle	Xiao *et al.* 2018
Y. lipolytica	Nervous necrosis virus (NNV)/Viral encephalopathy and retinopathy in marine fish	Capsid protein of red-spotted grouper nervous necrosis virus (RGNNV-CP)	Virus like particle	Luu *et al.* 2017

*VLPs are also purified protein(s).

purified from budding yeast was taken up by DCs and displayed by MHC class I molecules (Wadle *et al.* 2010). Recombinant *S. cerevisiae*-based vaccine expressing the T315I-mutated BCR-ABL antigen was able to reduce or eliminated BCR-ABL (T315I) leukaemia cells from the peripheral blood (Bui *et al.* 2010) without affecting normal BCR-ABL cells. Injection of heat-killed budding yeast expressing the tumour-associated antigen CEA induces CEA-specific immune responses, reduces tumour burden and extends overall survival in CEA-Tg mice (Wansley *et al.* 2008). BG from mutated budding yeast inhibits tumour metastasis *via* activation of macrophages and NK cells (Yoon *et al.* 2008). Whole recombinant budding yeast expressing brachyury protein allows activation and proliferation of brachyury-specific $CD4^+$ and $CD8^+$ T cells in vitro and these activated cells were able to kill tumour cells in absence of any obvious toxicity (Hamilton *et al.* 2013).

Rabbit anti-hCGβ-oHLα IgG generated by expression and purification of chimeric hCGβ-oLHα from budding yeast was able to inhibit the proliferation of hCG-expressing human colorectal cancer cells (LS-174, HCT-116, HCT-15 and KM-12) and also neutralise the bioactivity of hCG (Jiang *et al.* 2010). In another study, yeast cells were used for expression of melanoma protein for use as a prophylactic vaccine against a melanoma and despite the lack of information about epitopes recognised by MHC molecules and expressed protein was able to protect mice from tumour development (Riemann *et al.* 2007). B7–2(225)-scFv (FRP5) expressed and purified from *P. pastoris* yeast retains activity and localises to f ErbB2-expressing target cells was able to generate a costimulatory signal that results in enhanced proliferation of syngeneic T cells (Gerstmayer *et al.* 1997). Recombinant budding yeast expressing CEA acts as potential vaccine carrier and resulted in increased gene expression of IL-12, IFNγ and GM-CSF (Bernstein *et al.* 2008; Remondo *et al.* 2009). Different tumour antigens expressed in yeast are shown in Table 5.

After looking at some of the studies mentioned already, it can be concluded that expression of tumour-specific or tumour-associated antigens in yeast and the application of whole recombinant yeast is able to raise an immune response against a tumour with a high degree of specificity and reduce tumour burden and extended survival rate. Hence, yeast-based vaccine against tumour holds great value in cancer therapy and encourages further research.

WHOLE RECOMBINANT YEAST-BASED VACCINES

Although vaccination of individuals with a peptide-based vaccines (example Hepatitis B) is now routine practice throughout the world, still issues related to the stability of peptide-based vaccines and a special requirement for storage, transportation and shelve life warrants need for an alternative of peptide vaccines. So far, the cellular environment has been the most suitable for long-term stability and maintenance of protein structure, thus problem associated with stability of protein antigen can be overcome by expressing and the use of the whole cell.

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Table 2. Table showing list of various protozoan proteins expressed in various yeast species.

Yeast species	Source of antigen/Diseases	Antigen/Immunogen	Strategy	References
S. cerevisiae	*P. falciparum*/Malaria	Falc2.3, p195A, SERAI, Pfs25	Purified protein	Bathurst *et al.* 1993
		gp195	Purified protein	Chang *et al.* 1992
		Pfs25 surface protein	Purified protein	Kaslow and Shiloach 1994
		15 defined cytotoxic T lymphocyte (CTL) epitopes form plasmodium	Virus like particle	Gilbert *et al.* 1997
		MSP3	Purified protein	Hisaeda *et al.* 2002
		rMSP1(19)	Purified protein	Kumar *et al.* 2000
		TBV25H	NA	Noronha *et al.* 1999
		Pfs25 and Pfs28	Purified protein	Gozar *et al.* 1998
		Pfs25	Purified protein	Stowers *et al.* 2000
		MSP1	Purified protein	Stowers *et al.* 2001a
		TBV25H , Pfs25 EGF domains 1–4, $MSP1_{19,}$ $P30P2MSP1_{19}$	Purified protein	Stowers *et al.* 2001b
	P. vivax/Malaria	Circumsporozoite (VIVAX-1)	Purified protein	Collins *et al.* 1989
		Circumsporozoite (CSV) protein	Virus like particle	Vanloubbeeck *et al.* 2013
		Pvs25 and Pvs28	Purified protein	Sattabongkot *et al.* 2003
	T. gondii/Zoonosis toxoplasmosis	Microneme protein 16	Yeast surface display	Wang *et al.* 2018c
	P. yoelii/Malaria	MSP1(19)	Purified protein	Hirunpetcharat *et al.* 2003
P. pastoris	*P. falciparum*/Malaria	C-terminal region of the merozoite surface protein 1 (MSP119)	Purified protein	Zhang *et al.* 2006
		F2 domain of EBA-175	Purified protein	Yadava and Ockenhouse 2003
		MSP1–42	Purified protein	Zhang *et al.* 2002
		MSP-1(19), AMA-1 (domains I and II), AMA-1 + MSP-1(19), and fused AMA-1/MSP-1(19))	Purified protein	Arnot *et al.* 2008
		Apical membrane antigen 1 (PfAMA1)	Purified protein	Remarque *et al.* 2008
		19-kilodalton C-terminal fragment of merozoite surface protein 1.	Purified protein	Faber *et al.* 2007
	P. vivax/Malaria	AMA-1 and MSP-1	Purified protein	Rocha *et al.* 2017
		Ectodomain of apical membrane antigen 1	Purified protein	Vicentin *et al.* 2014
		MSP19	Purified protein	Soares and Rodrigues 2002
		PvCSP alleles (VK210, VK247, and *P. vivax*-like)	Purified protein	Gimenez *et al.* 2017
	P. berghei/Malaria	Circumsporozoite surface antigen	Yeast whole cell lysate	Jacob *et al.* 2017
	Babesia bovis/Bovine babesiosis	MSA-2a1, MSA-2b and MSA-2c	Purified protein	Gimenez *et al.* 2016
	T. con-golense/Trypanosomosis	Protease (congopain)	Purified protein	Boulangé *et al.* 2011
H. polymorpha	*P.falciparum*/Malaria	Circumsporozoite protein	Purified protein	Radosevic *et al.* 2010

With this intention, scientists focused their efforts in developing a whole recombinant yeast-based vaccine as pointed out by following recent studies. For example, dead whole recombinant budding yeast expressing Rv1886c (fbpB, mpt59, Ag85B) was able to activate antigen-specific T cells and were able in reducing mycobacterial population (Grover *et al.* 2016). Injection of even whole *S. boulardii* was able to raise humoral immune response (Hudson *et al.* 2016). Administration of killed whole recombinant yeast expressing mutant KRAS shows positive response in phase II of clinical trials (Chaft *et al.* 2014) while in another case injecting killed recombinant yeast expressing Hepatitis B virus protein was found safe and well tolerated in healthy subjects (Gaggar *et al.* 2014) and dead whole yeast was able to protect mice from pulmonary mucormycosis treated with diabetic ketoacidotic-steroid (Luo *et al.* 2014).

Recently it is observed that whole recombinant yeast producing RGNNV CP mount 9–27 times stronger anti-RGNNV CP IgG titers than purified RGNNV CP suggesting that whole recombinant yeast is a better and more efficient platform for antigen(s) delivery by oral immunisation (Kim *et al.* 2014) and heat-killed budding yeast was able to act against fungal infection (Liu *et al.* 2011). Many of the whole recombinant budding yeast-based

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Table 3. Table showing list of various bacterial proteins expressed in various yeast species.

Yeast species	Source of antigen/Diseases	Antigen/Immunogen	Strategy	References
S. cerevisiae	*A. pleuropneumoniae*/Pleuropneumoniae	ApxlA	Whole recombinant yeast (oral application)	Shin *et al.* 2003
		ApxIIA	Purified protein	Shin *et al.* 2005
		apxIA and apxIIA	Whole recombinant yeast (oral application)	Shin *et al.* 2007
	Bacillus anthracis/Anthrax	63-kDa form of Bacillus anthracis protective antigen	Purified protein	Robert *et al.* 2006
	M. tuberculosis/Tuberculosis	Rv1738, Rv2032, Rv3130, and Rv3841)	Purified protein	King *et al.* 2017
	L. monocytogenes/Listeriosis	Pore-forming protein listeriolysin O	Whole recombinant yeast	Walch *et al.* 2011
P. pastoris	*B. pertussis*/Pertussis	Bordetella pertussis pertactin (P69)	Purified protein	Romanos *et al.* 1991
	C. botulinum/Botulism	Rcombinant C-terminus heavy chain fragment from botulinum neurotoxin serotype C [rBoNTC(H(c))],	Purified protein	Dux *et al.* 2011
		Rcombinant H(C) fragment of botulinum neurotoxin, serotype A (rBoNTA(HC))	Purified protein	Potter *et al.* 2000
		Rcombinant H(C) fragment of botulinum neurotoxin, serotype A (rBoNTA(HC))	Purified protein	Potter *et al.* 1998
		N- and C-terminal subdomains of recombinant heavy chain fragment of *C. botulinum* neurotoxin serotype C	Purified protein	Huang *et al.* 2007
		Recombinant heavy chain fragment C of botulinum neurotoxin (BoNT/E)	Purified protein	Johnson *et al.* 2003
		Recombinant heavy chain fragment C of botulinum neurotoxin (BoNT/E)	Purified protein	Dux *et al.* 2006
	M. tuberculosis, Influenza A/Tuberculosis	Me2-HSP70 fusion protein	Purified protein	Ebrahimi *et al.* 2010
	M. tuberculosis/Tuberculosis	Recombinant heparin-binding haemagglutinin	Purified protein	Kohama *et al.* 2008
	Leptospira interrogans/Leptospirosis	LigANI	Purified protein	Hartwig *et al.* 2014

vaccines which are under different phases of clinical trials are revived elsewhere (Ardiani *et al.* 2010). Yeast cells were also able to activate OVA-specific $CD4^{+}$ T cells and both $CD8^{+}$ and $CD8^{-}$ DC cells were able to internalise yeast in equal amounts through dectin-1 (Backer *et al.* 2008). Oral administration of budding yeast expressing ApxIIA was able to mount an immune response against *A. pleuropneumoniae* (Shin *et al.* 2005, 2013) and recombinant yeast was also able to activate HBV X, S, and core-specific T cells and viral clearance (King *et al.* 2014). Thus, ample evidence is mentioned that showed the merits of using the whole recombinant yeast-based vaccines.

YEAST DISPLAY FOR VACCINES

Use of whole recombinant yeast expressing the immunogenic protein(s) from pathogenic species is an important and novel strategy. Still, yeast display (YD) or yeast surface display (YSD) appears more important and allows development of oral or edible vaccines. As a proof of concept, many recent reports showed the encouraging results where yeast display being used as a prophylactic vaccine. For example, an oral vaccine against candidiasis was introduced using molecular display systems with *S. cerevisiae* (Shibasaki and Ueda 2016). Identification of anti-B7-H4 antibodies using yeast-display library of recombinant antibodies derived from ovarian cancer patients was able to restore antitumor T cell responses (Dangaj and Scholler 2015). In another case, envelope protein E2 of bovine viral diarrhoea virus (BVDV) displayed on budding yeast surface was able to stimulate the production of CXCL-8 in macrophages. The same study also showed that the particulate nature of yeast is also important in efficient stimulation of T cells (Patterson *et al.* 2012). Feeding of freeze-dried budding yeast particles expressing the PCV2b Cap protein on their surface provides protection against PCV2b challenge in pigs (Patterson *et al.* 2015). Although yeast display is in its infancy stage and requires further research, some of the studies already

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Table 4. List of nematode and tick proteins expressed in yeast.

Yeast species	Source of antigen/Diseases	Antigen/Immunogen	Strategy	References
P. pastoris	*Schistosoma mansoni*/Intestinal schistosomiasis	Tetraspanin surface antigen protein (Sm-TPS2)	Purified protein	Cheng *et al.* 2013
		Sm14 antigen	Purified protein	Damasceno *et al.* 2017
	Necator americanus/Intestinal schistosomiasis	Glutathione S-transferase 1 (Na-GST-1)	Purified protein	Curti *et al.* 2014
		Glutathione S-transferase 1 (Na-GST-1)	Purified protein	Plieskatt *et al.* 2012
		Glutathione S-transferase 1 (Na-GST-1)	Purified protein	Zhan *et al.* 2010
	Boophilus microplus/Important in livestock diseases	Bm86 protein	Purified protein	Garci´a-Garci´a *et al.* 1998
		Bm86 protein	Purified protein	Garci´a-Garci´a *et al.* 2000
	Ascaris suum/Ascariasis in pigs	As16 protein	Purified protein	Wei *et al.* 2017
	Ancylostoma caninum/Intestinal related infection in dogs	Aspartyl protease inhibitor (API-1)	Purified protein	Delaney *et al.* 2005
	Ancylostoma ceylanicum/Intestinal related infection in mammals including humans	Aspartyl protease inhibitor (API-1)	Purified protein	Delaney *et al.* 2005

Table 5. List of tumour associated antigens expressed in yeast.

Yeast species	Antigen/Immunogen	Tumour type	Strategy	Clinical phase	Reference
S. cerevisiae	Brachyury (GI-6301)	Advanced malignant solid Tumours (chordoma)	Whole recombinant yeast	Phase-I	Heery *et al.* 2015
	Brachyury (GI-6301)	Epithelial-mesenchyma	Whole recombinant yeast	Phase-I	Hamilton *et al.* 2013
	Cancer testis antigen NY-ESO-1	Melanoma	Yeast surface display	Phase-I	Mischo *et al.* 2011
	Human MART-1	Melanoma	Whole recombinant yeast	*Preclinical	Riemann *et al.* 2007
	Derived Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Melanoma	Purified protein	Phase-III	Lawson *et al.* 2015
	KRAS	Lung adenocarcinoma	Whole recombinant yeast	Phase-II	Chaft *et al.* 2014
	CEA	Carcinoma	Whole recombinant yeast	Phase-I	Bilusic *et al.* 2014
	Human MART-1 (hMART-IT)	Melanoma	Whole recombinant yeast	*Preclinical	Tanaka *et al.* 2011
	BCR-ABL^{T315I}	Leukemias	Whole recombinant yeast	*Preclinical	Bui *et al.* 2010
	Single chain chimeric peptide composed of hCGβ and oLHα	NA	Purified protein	*Preclinical	Jiang *et al.* 2010
	Human CEA (yeast-CEA	NA	Whole recombinant yeast	*Preclinical	Wansley *et al.* 2008
	NY-ESO-1-Aga2p	NA	Purified protein	*Preclinical	Wadle *et al.* 2010
P. pastoris	HPV16 L1 antigen	Papilloma	Whole recombinant yeast	*Preclinical	Bolhassani *et al.* 2014

*Preclinical indicate that experiments were performed in model organisms and not in any human subjects.

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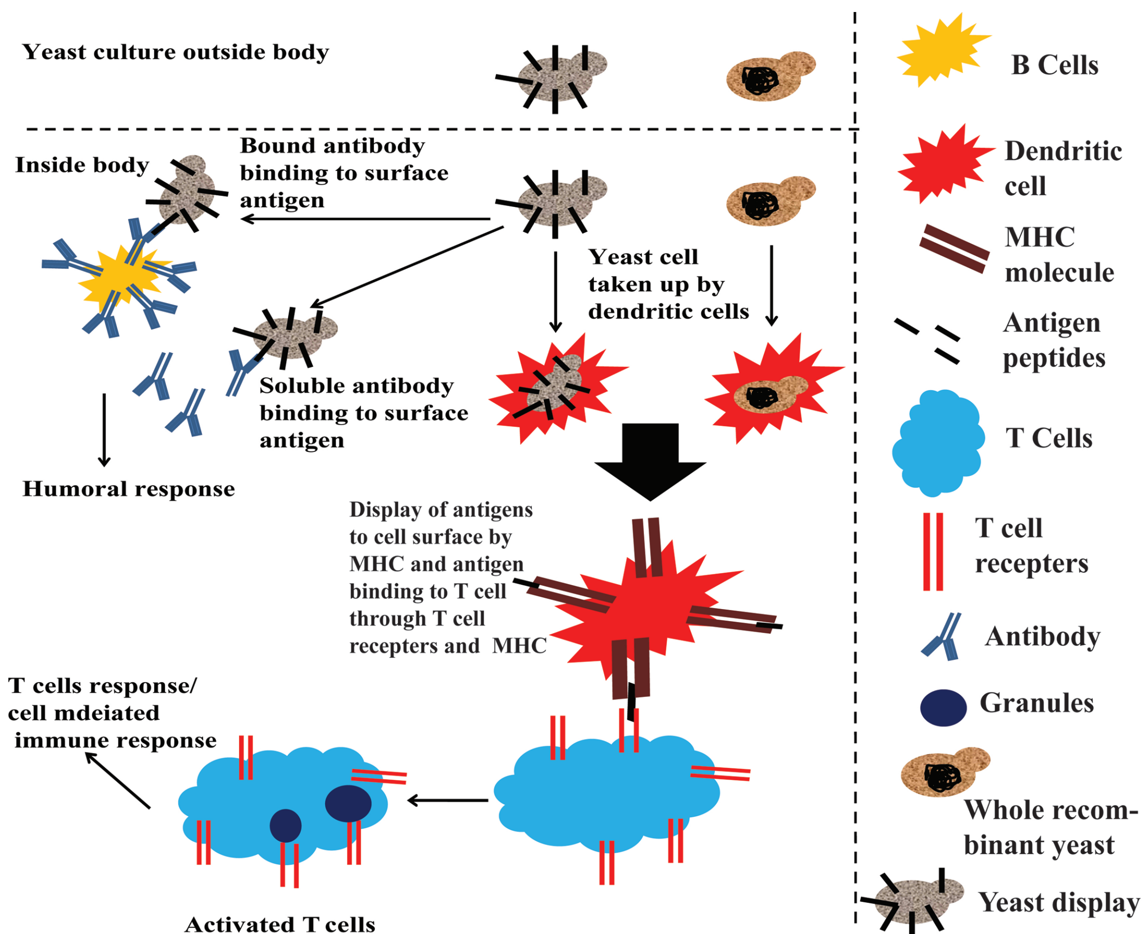

Figure 2. Schematic showing suitability of whole yeast and yeast display in generating an immune response. Note as a proof of concept, the detailed mechanism of induction and interaction of humoral and cell-mediated immune response is not shown in the figure.

highlighted in this article clearly showed the impact this technology may have on vaccine development and application.

YEAST-DERIVED VLPS (VIRUS-LIKE PARTICLES) AS VACCINES

Although purified protein antigen proved to be of high value in inducing a protective immune response and as a result, several protein immunogens expressed and purified from yeast have already reached clinical trials. But limitations associated with purified protein including their low immunogenicity (due to lack of particulate nature), small size, rapid body clearance means an alternative need to be looked which can overcome the issues highlighted above (Purcell, McCluskey and Rossjohn 2007; Aguilar and Rodríguez 2007). This led to the development of VLPs which resembles like a virus but lack viral genome. Use of these self-assembled protein complexes as vaccines proved to be much superior compared to purified protein immunogens. Because of their particulate nature VLPs can raise both B and T cell immune responses (Akahata *et al.* 2010). VLPs can provide a safer alternative to attenuated viruses. VLPs were used to develop FDA-approved vaccines for Hepatitis B and human papillomavirus (Zhang *et al.* 2015b). This strategy allows expressed protein antigens to assemble into VLPs (Kingsman *et al.* 1995), and involves fusion of protein or antigen of interest with yeast Ty retrotransposon. The chimeric fusion protein can self-assemble like virus particles without compromising their immunogenic nature. Apart from that the p1 and Gag 1 protein of *S. cerevisiae* transposon Ty1 can be used for the construction of bi-functional VLPs (Marchenko *et al.* 2003; Powilleit, Breinig and Schmitt 2007). List of protein antigens that were expressed in yeast to produce VLPs is shown in Tables 1–3. The detailed account of VLPs fall outside the scope of present review and same can found elsewhere (Roldao *et al.* 2010; Mohsen *et al.* 2017).

YEAST CELL WALL COMPONENT AS AN IMMUNE MODULATOR

Immunogenic or inherent adjuvant nature of yeast cells is attributed to its cell wall components. Initially, it was believed that polysaccharides, particularly glucans of cell wall act as a natural adjuvant and this generalisation is well supported by several studies that showed that BGs acts as an immune stimulant or modulator (Pillemer and Ecker 1941; Chihara *et al.* 1969) and BGs were also known for their protective role against microbial infection and cancer (Brown and Gordon 2003; Borchers *et al.* 1999). Detail account of BGs and description of their immune modulatory nature is beyond limits of present review and can be found elsewhere (Vetvicka 2011). Suffice to say that carbohydrates or polysaccharides components, example glucans confer important natural adjuvant nature to yeast cells that are proving highly valuable in using whole yeast or yeast display as an important strategy in developing yeast-based vaccines. An example, BGs was able to induce production of cytokines and

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NFkB in BMDM cells (Walachowski, Tabouret and Foucras 2016). A recent study shows that β-(1→6)-glucans was completely phagocytosed by DCs and helps in mounting immune response against fungal response (Noss *et al.* 2015) and oral administration of BGs was able in clearance of Hepatitis B virus in a mouse model (Yu *et al.* 2015). BGs were also found to activate and enhances CTL responses through the PI3K pathway (Ding *et al.* 2015) and were also able to provide protection from systemic aspergillosis (Clemons *et al.* 2014). BGs from budding yeast were able to induce the expression of several cytokines (IL-10, TGF-β1 and IL-2) and a tolerogenic enzyme (IDO) in bone marrow-derived DCs as well as spleen cells (Karumuthil-Melethil *et al.* 2014). These studies clearly showed that BGs of yeast cell wall act as important stimulators and modulators of immune response that have definite and important implication in vaccine development and application.

CONCLUDING REMARK

During last two decades more than 500 publications appeared (Pubmed: http://www.ncbi.nlm.nih.gov/pubmed) in which yeasts were used for the expression of heterologous proteins to raise an immune response against different pathogenic species and tumour (Jung *et al.* 2010) and the pace with which information is gathering in this emerging field make it difficult to compile all the work together and can be found elsewhere (Ardiani *et al.* 2010; Shin and Yoo 2013). Out of so many yeast species mentioned in this review, *S. cerevisiae* and *P. pastoris* emerge as a most promising candidates for expression of heterologous proteins for vaccines development. Apart from non-pathogenic and inherent adjuvant nature, the ability of proteins expressed in yeast to raise an immune response and further development of whole recombinant or yeast display strategies qualify them for the expression of the heterologous protein for development of oral or edible vaccines. These novel strategies of using whole recombinant or yeast display provide numerous benefits in vaccine development including cutting down the cost associated with protein purification, increased stability of antigens in cellular environment compared to purified proteins in a buffer, unlike peptide-based vaccines whole recombinant yeast-based vaccines does not require special adjuvant, ease, and economics associated with mass culture of recombinant yeast compared to other systems like mammalian cells or plant system makes yeasts a model of choice for vaccine development. Although numerous studies are available, which showed the importance and benefits of application of YD or WRY as a vaccine; only a few studies are available that investigate the stability of expressed heterologous protein antigen in WRY. For example, it is recently shown that in stationary phase yeast cells, expressed protein antigen or immunogen remains stable for up to a year when stored at 2–8°C and at 23–25°C (Kumar 2018). While another study investigates the stability of protein antigen in WRY over a period of six months (Wang *et al.* 2018d).

The yeast display strategy helps in the quick and fast recognition of pathogen antigens and particulate nature of yeast (whole recombinant yeast and yeast disply-based vaccines) helps in the engulfment of whole cells whose components are then displayed on the surface of APCs, which help in activation of T cells through interaction and recognition of antigen peptides through MHC molecules. Activation of T cells is important for a cell-mediated immune response, in mounting the full humoral response and for a secondary immune response. Apart from that yeast display also provide an opportunity that antigen on yeast cells can be directly recognised by soluble antibodies in blood or bound to B cell surface. Suitability of whole recombinant and yeast display in the activation of an immune response is shown in Fig. 2.

It is also interesting that the yeast-based vaccines are also able to modulate tumour growth and developments in this field are encouraging that provide required and necessary imputes for further studies. The ability of cell wall components, including beta-glucan (BG) to activate macrophages and APCs, or DCs may be important in checking fungal infection due to the conserved nature of cell wall components across fungal species.

The fact that many yeasts-based vaccines are under different phases of clinical trials and many of them reached to the third phase of clinical trials showed clear merits associated with these vaccines and use of protein purified protein from yeast as a peptide vaccine against hepatitis B is an important success story of a yeast-based vaccine. Thus, in the end, it can be concluded that further identification of more protein antigens, their expression in yeast and the use of whole recombinant or yeast display will certainly prove instrumental in cutting time and cost involved in vaccine preparation. These yeasts-based vaccines will be of great value in vaccine development, particularly in those cases where the mass culture of associated pathogens is still not feasible, example leprosy, malaria. Thus, yeast-based vaccines provide a ray of hope for both in public health and for farm animals, and we can hope that in near future yeast-based edible or oral vaccine may be common on shelves in medical dispensaries or clinical stores.

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