Review Article

Pichia pastoris an Ideal Host for the Production of Recombinant Influenza Vaccines

Amir Asghari Baghkheirati[1], Mohammad Hadi Sekhavati[2], Seyed Mostafa Peighambari[1], Kiarash Ghazvini[3], Jamshid Razmyar[1*]

1. Department of Avian Health and Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Use your device to scan and read the article online

How to Cite This Article Asghari Baghkheirati, A., Sekhavati, M. H., Peighambari, S. M., Ghazvini, K., & Razmyar, J. (2024). *Pichia pastoris* an Ideal Host for the Production of Recombinant Influenza Vaccines. *Iranian Journal of Veterinary Medicine, 18*(4), 465-478. http://dx.doi.org/10.32598/ijvm.18.4.1005523

http://dx.doi.org/10.32598/ijvm.18.4.1005523

ABSTRACT

Pichia pastoris is a methylotrophic yeast with remarkable characteristics such as lacking endotoxin, producing high amounts of recombinant protein, performing post-translational modifications, and so on. Influenza A virus, a member of the Orthomyxoviridae family, is the cause of avian influenza. Three avian influenza virus subtypes, H5, H7 and H9, are commercially and physiologically significant in the poultry industry. Some researchers considered influenza to be the next pandemic disease. Nowadays, researchers have paid attention to producing novel and effective recombinant vaccines, especially in the poultry industry. Due to the advantages of *P. pastoris* yeast, it can be used as an ideal expression system for producing subunit vaccines. Although several studies have been conducted in this field, there is no comprehensive review of using *P. pastoris* to produce recombinant influenza vaccines. This review explains the different strains, phenotypes, and advantages of this yeast and then the production of recombinant influenza vaccines using this expression system is discussed in detail.

Keywords: Influenza, *Pichia pastoris*, Recombinant, Vaccine, Veterinary medicine

Article info:
Received: 25 May 2023
Accepted: 10 Jul 2023
Publish: 01 Oct 2024

*** Corresponding Author:**
Jamshid Razmyar, Associate Professor.
Address: *Department of Avian Health and Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+98 (21) 61117000*
E-mail: *jrazmyar@ut.ac.ir*

Introduction

Recombinant proteins are frequently made by yeasts, which are unicellular fungi. *Pichia pastoris* and *Saccharomyces cerevisiae* are two well-known yeast systems that can be used for this purpose. The ability of yeast systems to carry out post-translational modifications such as acetylation, phosphorylation, glycosylation, proper protein folding, and the absence of endotoxin are among its advantages (Tanaka et al., 2012; Kuruti et al., 2020; De et al., 2021).

P. pastoris is a methylotrophic organism known as an ideal organism for expressing recombinant proteins on an industrial scale (Alizadeh et al., 2013; Barone et al., 2023; De et al., 2021). *P. pastoris* can use methanol as its only carbon source. During the oxidation process inside the peroxisome, this yeast utilizes the alcohol oxidase enzyme to metabolize methanol (Maleknia et al., 2011; Athmaram et al., 2011; Moridi et al., 2020). Different strains of this yeast have been used to produce recombinant proteins (Mohammadzadeh et al., 2021). It should be noted that all *P. pastoris* strains, such as auxotrophic mutants (GS115) and protease-free strains (SMD1163, SMD1165 and SMD1168), are derived from the wild strain NRRL-Y 11430 (Tanaka et al., 2012). Influenza A virus, a member of the Orthomyxoviridae family, is the cause of avian influenza. Three avian influenza virus subtypes, H5, H7 and H9N2, are commercially and physiologically significant in the poultry industry (Gholami et al., 2022; Mirzaie et al., 2021; Mohammadi et al., 2021; Abtin et al., 2022). Some researchers considered influenza the next pandemic (Morens et al., 2023). Approximately between 250000 and 500000 individuals die from influenza virus infections worldwide annually (Norouzian et al., 2014; Perdue & Swayne, 2005; Kim et al., 2022).

Avian influenza subtype H9N2 is the most prevalent influenza virus in poultry worldwide. It imposes economic losses on the poultry industry and has zoonotic potential (Alizadeh et al., 2009; Mirzaie et al., 2020; Zhao et al., 2021; Golgol et al., 2023). Nili and Asasi, (2003) demonstrated mortality rates between 20% and 60% on H9N2-infected farms. One possible explanation for this high mortality rate is co-infection with other respiratory diseases.

The expression of the recombinant protein and subsequent manufacturing of the vaccine in yeast are more suitable in terms of timing and scale of production than insect, mammal, or *Escherichia coli* expression systems (Athmaram et al., 2011). Genetic engineering technology and veterinary medicine allow us to create novel and effective recombinant vaccines against various diseases such as brucellosis, Clostridium, influenza, tuberculosis, and so on (Soleimanpour et al., 2015; Nouri Gharajalar et al., 2016; Mayahi et al., 2016; Yousefi et al., 2016; Farsiani et al., 2016; Shirdast et al., 2021; Asghari Baghkheirati et al., 2023; Taghizadeh & Dabaghian, 2022). Besides, new subunit vaccines have been made in medicine against SARS-CoV-2, enterovirus, papillomavirus, malaria, etc. using the *P. pastoris* expression system (Mukhopadhyay et al., 2022; Xu et al., 2023; Noseda et al., 2023; Kingston et al., 2023; Li et al., 2023). Previous literature has emphasized the use of this yeast as a safe, cost-effective and suitable organism for vaccine production in the healthcare industry (Kuruti et al., 2020; Barone et al., 2023; De Sá Magalhães & Keshavarz-Moore, 2021). This review study aims to describe *P. pastoris* as one of the most efficient expression systems for developing recombinant vaccines for the poultry industry, focusing on avian influenza vaccines.

P. pastoris phenotypes

Depending on the yeast genotype, the presence or absence of the alcohol oxidase genes (*AOX1* and *AOX2*), and the use of methanol, this yeast can be classified into three phenotype categories (Maleknia et al., 2011; Singh & Narang, 2020). Although both genes affect the production of enzymes and consumption of methanol, the alcohol oxidase 1 promoter has a greater impact.

1) Mut+phenotype (X33 and GS115 strains): This group is the natural yeast *P. pastoris* with *AOX1* and *AOX2* genes. Compared to the other two phenotypes, these strains use methanol more quickly, consume more oxygen, and express more recombinant protein. For these reasons, most studies have used them with this phenotype as an industrial strain (Cámara et al., 2017; Singh & Narang, 2020).

2) Muts phenotype (KM71 strain): Although the *AOX2* gene is present in this group, the *AOX1* gene has been eliminated. Due to the deletion of *AOX1*, these stains cannot be used quickly by methanol. Since these strains use methanol slowly, more complex proteins will have time to acquire their correct conformation before being secreted into the medium (Wollborn et al., 2022).

3) Mut-phenotype (MC100-3 and MC101-1 strains): In this group, both *AOX1* and *AOX2* promoters have been deleted, so these strains cannot use methanol and are practically unable to grow in

an environment containing methanol. The main carbon sources utilized by these strains are glycerol, sorbitol, or mannitol (Singh & Narang, 2020).

The advantages of using *P. pastoris*

Several reasons exist for using this yeast as an expression system (illustrated in Figure 1 and explained here).

1) Ease of working: There is no need to have complex culture media or special nutrients for *P. pastoris* yeast propagation. This yeast can be grown easily using a culture medium containing yeast extract, peptone and dextrose (Kuruti et al., 2020).

2) High cell density: Fermentation is an essential process for recombinant protein production, and its efficiency is highly dependent on cell density. *P. pastoris* can reach a high cell density in an optimized culture medium and produce more recombinant antigens than other expression systems (Zhang et al., 2020).

3) Eukaryotic expression system: Compared to prokaryotic systems, *P. pastoris* is a eukaryotic organism that can produce mammalian and avian proteins more similar to their original form (Kuruti et al., 2020).

4) Genomic integration of the desired gene: The desired gene can be integrated into several locations of the yeast chromosome. This characteristic plays an important role in the stability of the gene and increased production of the influenza protein (Wu et al., 2023).

5) High efficiency in recombinant protein production: One of the reasons for the tendency towards this yeast is its high expression level. The recombinant protein produced by this yeast can include more than 80% of the total proteins in the culture medium (Li et al., 2007). The *AOX1* promoter, one of the most potent eukaryotic promoters, has been used to produce a variety of recombinant proteins, with documented yields of up to 20–30 g/L (Tanaka et al., 2012).

6) Post-translational modifications: One of the most important processes in protein synthesis, performed after transcription and translation, is glycosylation. The role of glycosylation in protein folding, protein structural stability, specific signal transmission, and secretion processes has been proven. In comparing *P. pastoris* and *S. cerevisiae*, it should be stated that the oligosaccharide chains that are attached to proteins and make glycoproteins are more reliable in *Pichia* (Li et al., 2007). One of the advantages of using *P. pastoris* yeast is the lack of mannosyltransferase. This enzyme causes the production of α-1, 3-mannosyl bonds, which is seen in *S. cerevisiae*. These connections differ from those in the mammalian system and may be recognized and rejected by the human immune system. On the other hand, *P. pastoris* yeast is a better option than *S. cerevisiae* for producing a recombinant protein because it has a higher capacity for producing heavy proteins and secretes fewer unwanted internal proteins into the extracellular environment (Tanaka et al., 2012).

7) Probiotic properties: Several investigations have been accomplished regarding this yeast's probiotic features. It was demonstrated that the X-33 strain can survive in food at an appropriate concentration for at least two months. *Salmonella* spp., *Clostridium* spp. and *E. coli* are among the most important bacterial pathogens in the poultry industry that cause significant economic losses (Seyedtaghiya et al., 2021; Daneshmand et al., 2022; Peighambari et al., 2023). *P. pastoris* can be a probiotic and antibiotic alternative to prevent and control these pathogens. This yeast administration prevented *Salmonella typhimurium*'s growth in the culture medium and decreased bacterial colonization in the BALB/c mice intestine (Franca et al., 2015). The mice had a higher survival rate in the challenge test with the acute strain of *S. typhimurium* (50% to 80%) than the control group (20% to 50%). In another study, Gaboardi et al. (2019) found that the administration of *P. pastoris* X-33 strain in the quail's diet could increase egg weight, adjust the immune system, and increase the level of antibodies against infectious bronchitis virus (IBV), Newcastle disease (ND) and infectious bursal disease (IBD), compared to the control group. Transgenic or wild-type *P. pastoris* strains can be used as probiotics in chickens as antibiotic alternatives to control necrotic enteritis (SGil de Los Santos et al., 2018; Kulkarni et al., 2022).

8) Natural adjuvant activity: It has been demonstrated that the yeast cell wall components have inherent adjuvant properties (Franca et al., 2015). In other words, yeast-based vaccines do not need adjuvants like aluminum to stimulate the immune system (Stubbs et al., 2001). Therefore, when administered, expressed recombinant proteins and yeast cell wall components will be more immunogenic (Wasilenko et al., 2010; Asghari Baghkheirati et al., 2023).

P. pastoris transformation

It has been indicated that multiple copies of the desired gene in the *P. pastoris* genome result in elevated gene expression. Therefore, it is important to choose the best

Table 1. Using *P. pastoris* in the production of influenza subunit vaccines

Influenza Subtype	Antigen	*P. pastoris* Strains	Vector	In Vivo Test	Adjuvant	Result	Ref.
H3N2 (A/Victoria/3/75)	NA	GS115	pPIC9	BALB/c mice, SC, 3 times	1st injection: Ribi adjuvant Booster injections: *S. typhimurium* monophosphoryl lipid A.	50% Protection against a lethal challenge	Martinet et al., 1997
H3N2 (A/Victoria/3/75)	HA	GS115	pPIC9	BALB/c mice, SC,3 times	1st injection: Ribi adjuvant; booster injections: Monophosphoryl lipid A and muramyl dipeptide	Complete protection against a lethal challenge	Saelens et al., 1999
H1N1 (A/NewCaledonia/20/1999) & H3N2 (A/Wisconsin/67/2005)	M2e	P. pastoris	pPICZα	BALB/c mice, IP, 3 times	Freund's adjuvant (CFA/IFA)	Significant protection against lethal challenge with an H1N1 or H3N2 viruses	Mu et al., 2016
H5N1 (A/Egret/Hong Kong/757)	HA	GS115	pPIC9K	Chicken Oral gavage	None	Production of virus-neutralizing antibodies.	Wasilenko et al., 2010
H5N1 (A/swan/Poland/305-135V08/2006)	HA	SMD 1168 and KM 71	pPICZαC	BALB/c mice, 3 times intradermal injection	Monophosphoryl lipid a & synthetic Trehalose Dicorynomycolate in Tween® 80	Eliciting a high immune response (512 HI titer)	Kopera et al., 2014
H5N1 (A/CK/HK/Yu22/2002)	HA1	GS115	pPIC9K	BALB/c mice, SC, 3 times	Freund's incomplete adjuvant	The HI titer of the anti-rHA1 serum was calculated as 1280.	Lin et al., 2016
H5N1 (A/swan/Poland/305-135V08/2006)	HA	KM 71	pPICZαC	Chicken, SC, twice	Aluminum hydroxide	Protection from lethal challenge.	Pietrzak et al., 2016
H5N1 (A/swan/Poland/305-135V08/2006)	HA	KM 71	pPICZαC	Chicken, SC, twice.	Alhydrogel	Eliciting a strong humoral response	Stachyra et al., 2017
H5N1 (A/Hatay/2004)	HA	SMD1168	pPIC9	Chicken, SC, twice	Freund's adjuvant	HI values of up to 7 log2	Nguyen et al., 2014
H5N1 (A/Hatay/2004)	HA	P. pastoris	pPICZαC	Mice, SC, 3 times	2% aluminum hydroxide	A maximum of 280 HI titer	Murugan et al., 2013
H5N1 (A/Hatay/2004)	HA & M1	GS115	pPICZαC	Swiss albino mice	2% aluminum hydroxide	HA titer of 1:32	Subathra et al., 2014a
H5N1 (A/Hatay/2004)	NA	GS115	pPICZαC	Mice, SC, 3 times	2% aluminum hydroxide	Significant immune response against rNA. (ELISA).	Subathra et al., 2014b
H1N1 (A/newCaledonia/20/99)	NA (low-glycosylated NA)	GS115	pPIC9	BALB/c mice, IP, 3 times	Aluminum hydroxide	High antibody titer (1:4,900)	Yang et al., 2012
H1N1 (A/Brisbane/59/2007)	HA	KM71H	pPICZαA	BALB/c mice, IM, 3 times	Aluminum hydroxide	Broad protection in mice.	Wang et al., 2019

Influenza Subtype	Antigen	*P. pastoris* Strains	Vector	In Vivo Test	Adjuvant	Result	Ref.
H1N1 (A/H1N1/Gdansk/036/2009)	HA	KM 71	pPICZαA	BALB/c mice, SC, 3 times	Alhydrogel (aluminum hydroxide)	HI titres as high as 1: 2048	Kopera et al., 2019
H1N1 (A/California/04/2009)	HA	GS115	pPIC9K	Rabbits and mice, IM, twice	1st injection: Freund's complete adjuvant (FCA). Booster: Freund's incomplete adjuvant (FIA)	Mean HI titers of 1:32	Athmaram et al., 2011
H7N9 (A/Hangzhou/1/2013)	HA	*P. pastoris*	pPICZαA	BALB/c mice, IM, twice	Aluminum hydroxide	High antibody titer & complete protection	Liu et al., 2020

method for efficient transformation. The most efficient way to transform *P. pastoris* is to use the settings of 25 μF, 200 Ω, and 1500 V for the instrument's capacitance, resistance, and voltage, respectively (Wu & Letchworth, 2004; Yongkiettrakul et al., 2009; Sulfianti et al., 2015; Pratanaphon et al., 2018). Furthermore, pretreating yeast cells with lithium acetate and dithiothreitol has been shown to boost transformation efficiency significantly 150-fold (Wu & Letchworth, 2004).

P. pastoris vectors

There are two expression vectors for *P. pastoris*, including pPIC9k and pPICZα (A, B and C). The only difference between pPIC9 and pPIC9K is the kanamycin resistance gene, which gives *Pichia* resistance to Geneticin®. As the number of integrated copies increases, *Pichia* becomes resistant to higher concentrations of Geneticin® and the expression level will be higher. pPICZ A, B and C are 3.3-kb expression vectors that express recombinant proteins in *P. pastoris*. This vector's multiple cloning sites in three reading frames (A, B and C) make it easier to clone the desired gene in a frame with the C-terminal peptide containing a polyhistidine (6xHis) tag and the c-myc epitope. The characteristics of pPIC9k (Invitrogen, Catalog No. V175–20) and pPICZα vectors (Invitrogen, Catalog No. V190-20) are shown in Figure 2.

P. pastoris usage in the production of avian influenza vaccine candidates

The development of influenza vaccines primarily focuses on the hemagglutinin (HA) protein, the main antigenic protein of the influenza virus. Therefore, most research investigations have focused on selecting HA epitopes and their production in various *P. pastoris* strains. Researchers have employed *P. pastoris* yeast to produce various recombinant proteins, including influenza antigens (Sulfianti et al., 2015; Qian et al., 2021).

Table 2. Using *P. pastoris* for cloning and expression of influenza antigens

Influenza Subtype	Antigen	*P. pastoris* Strains	Vector	Methanol Concentration (V/V)	Ref.
H1N1 (A/Jakarta/271/2011)	HA	GS115	pPICZα-A	1%	Sulfianti et al., 2015
H5N1 (A/Thailand/3(SP-83)/2004)	HA2	GS115	pPICZα-A	1% every 12 h	Pratanaphon et al., 2018
H5N1 (A/Viet Nam/DT-036/2005)	NA (N1 head domain)	KM71	pPICZα-A	2-3% (96 h)	Yongkiettrakul et al., 2009
H5N1 (A/chicken/5858/Malaysian 2004)	NS1	GS115	pPICZαA	Not mentioned	Abubakar et al., 2011
H1N1 (A/California/07/2009)	HA2 and NP	*P. pastoris*	pPICZαB	1% for 96 h	Rungrojcharoenkit et al., 2020
H1N1 (A/PR/8)	PB1-PB2-PA	KM71	pPicZaA & pPic9	0.5% every 24 h for 96 h	Hwang et al., 2000

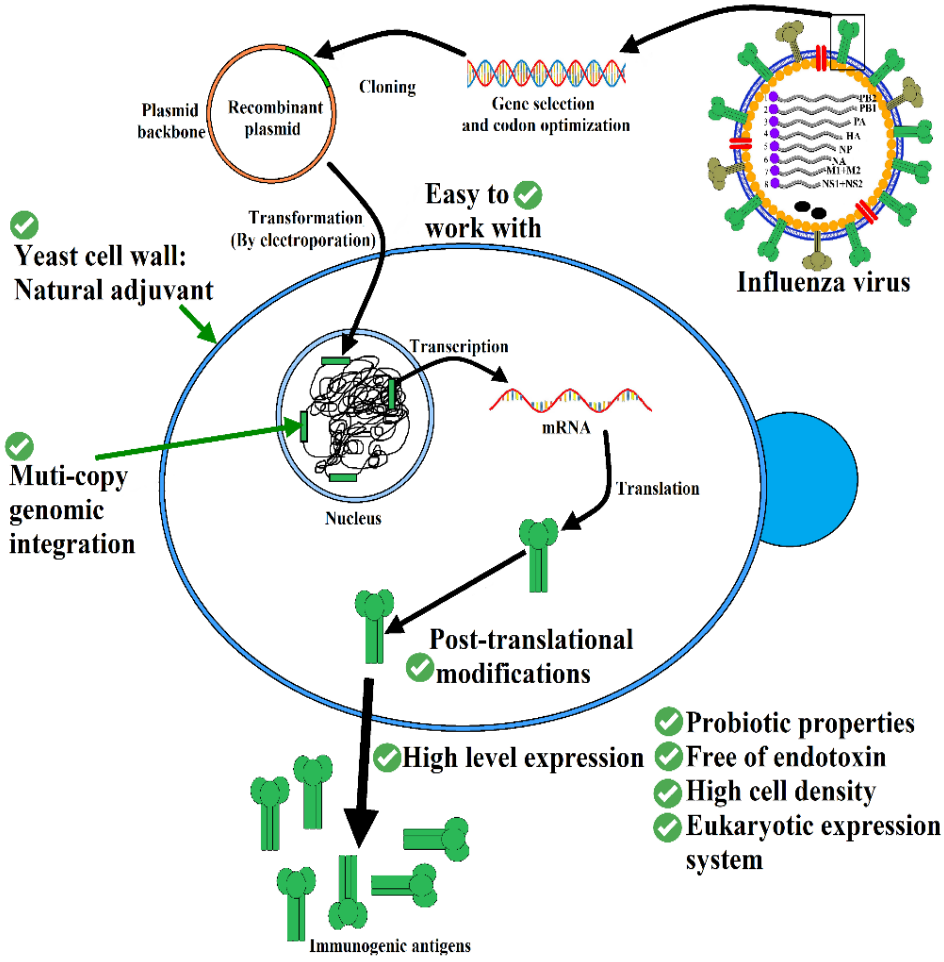

Figure 1. Advantages of using *P. pastoris* in recombinant expression of influenza antigens according to the in vitro studies

Notes: The trimeric hemagglutinin antigen, the most important antigen in stimulating the immune system, was shown as a sample.

Some scientists delivered these proteins through injections or oral administration to animal models, mainly mice and chickens and then measured the antibody titer. Many investigations have been conducted on the various aspects of influenza virus transmission, clinical symptoms, virology, serology, and the development of a novel vaccine using genetic engineering (Salamatian et al., 2020; Mirzaie et al., 2021; Mohammadi et al., 2021; Sahebnazar et al., 2021).

Several studies indicated that subunit influenza vaccines produced using the *P. pastoris* expression system can elicit high antibody titers in mice and chickens (Taghizadeh & Dabaghian, 2022; Asghari Baghkheirati et al., 2023). For instance, Pietrzak et al. (2016) transformed two hemagglutinin proteins, one with a cleavage region sequence (H5DH) and one without it (H5DHΔ), in *P. pastoris*. The recombinant antigens were diluted in PBS and injected subcutaneously in the neck area of SPF Leghorn laying hens twice. It was found that 100% of the chickens injected with H5DHΔ had high titers of neutralizing antibodies in the HI assay. Interestingly, all vaccinated chickens survived the challenge with H5N1, and no clinical symptoms were observed, but the control group chickens died on the fourth day after the challenge. This study shows that using the yeast system to produce recombinant proteins as a subunit vaccine can effectively protect chickens against lethal challenges. In research conducted by Liu et al. (2020), the complete *HA* gene of the H7N9 subtype (A/Hangzhou/1/2013) was cloned into the pPICZαA plasmid. Then, the resulting recombinant plasmid was linearized by the BglII restriction enzyme and transformed into *P. pastoris* using the electroporation technique. Recombinant H7 protein led to immunostimulation, high HI titer and 100% protection of mice following challenge with wild virus. Wasilenko et al. (2010) cloned the *HA* gene of the A/Egret/Hong Kong/757.2/02

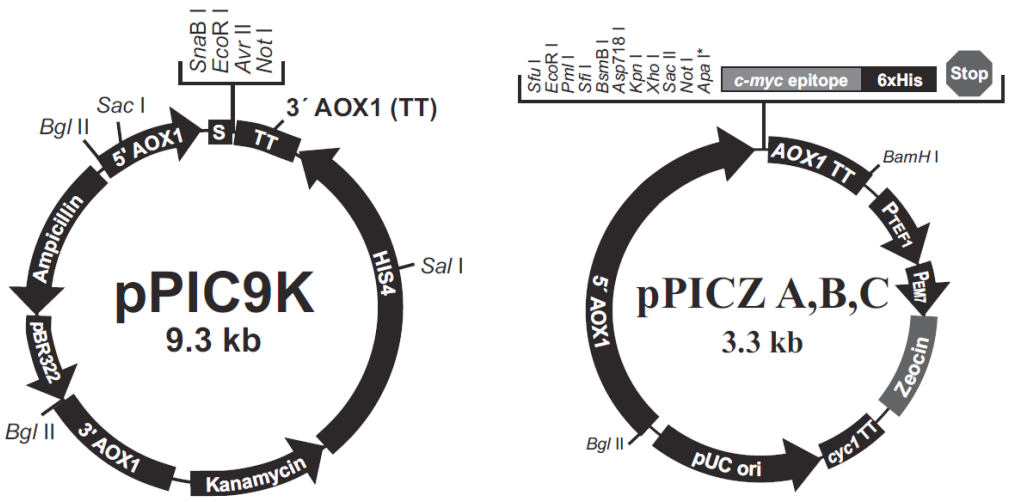

Figure 2. The features of the pPIC9K and pPICZ A, B and C vectors (invitrogen)

(H5N1) strain along with alpha-agglutinin as an anchor into the pPIC9K plasmid. The resulting recombinant plasmid was transformed into the *P. pastoris* GS115 strain. It was found that the recombinant vaccine can agglutinate red blood cells in the HA test, which indicates the yeast's correct production of HA protein. In addition, the oral administration of the vaccine to SPF Leghorn chickens resulted in the production of neutralizing antibodies. Nguyen et al. (2014) used the HA1 sequence and cloned it into the pPIC9 vector. They transformed the *P. pastoris* SMD1168 strain and administered the obtained recombinant antigens into BALB/c mice and chickens. The vaccine produced a high antibody titer in the HI test (6.7 and 7 titers in mice and chickens, respectively). According to reports, M1, one of the main structural proteins in influenza viruses with protected epitopes, can stimulate CD8+ lymphocyte cells and protect chickens against influenza infection and mortality. It is possible to produce multiple subunit antigens using the yeast expression system. During the study of Subathra et al. (2014a), the sequence of M1 and HA genes was obtained from the A/Hatay/2004/H5N1 strain and cloned in the pPICZαC plasmid and transformed into the *P. pastoris* GS115 strain. Based on their results, HA and M1 proteins can be combined to make faster and less expensive vaccines for influenza. In another study, Ebrahimi et al. (2010) used the KM71H strain and the pPICZαA plasmid to produce the M2e antigen of the H9 subtype. They demonstrated that the subcutaneous injection of antigen could produce polyclonal antiserum in rabbits.

Moreover, the expressed antigen could also be used to produce commercial ELISA kits. Shehata et al. (2012) prepared an ELISA kit using the *P. pastoris* GS115 strain to detect H5 influenza infection. The results showed that rHA1-ELISA has high specificity and sensitivity. Studies related to recombinant influenza vaccine production, with and without in vivo tests, were illustrated in Tables 1 and 2, respectively.

P. pastoris usage in the production of other recombinant vaccines

In addition to influenza vaccines, there are so many studies in which researchers have produced recombinant antigens. Several studies used *P. pastoris* to express *Mycobacterium tuberculosis* as a novel tuberculosis vaccine candidate and the results of their studies showed that this vaccine could elicit protective immunity in BALB/c mice (Mosavat et al., 2016; Kebriaei et al., 2016; Ravansalar et al., 2016).

In the study of Zhang et al. (2015), one of the outer membrane proteins of Proteus mirabilis called OmpA was expressed in *P. pastoris* and a high level of protection (80%) was observed in administered chickens. It has been documented that chickens vaccinated with the recombinant reticuloendotheliosis vaccine, produced by the SMD1168 strain, were completely protected against challenge with REV (Li et al., 2012). Oral administration of transgenic *P. pastoris* cells containing VP2 protein can cause a high level of protection against IBD in chickens (Taghavian et al., 2013). Yeast expression systems have been used in different studies to produce Eimeria (EtMic2) and avian reoviruses (σC and σB) proteins (Zhang et al., 2014; Yang et al., 2010). Furthermore, this strong expression system has been used for recombinant production of antimicrobial peptides that can be considered as antibiotic alternatives (Neshani et al., 2018; Neshani et al., 2019; Ghazvini et al., 2021; Azghandi et al., 2022).

Discussion

Influenza is one of the most crucial diseases that has resulted in uncompensated losses to the poultry industry worldwide (Nili & Asasi, 2003; Golgol et al., 2023). Today, inactivated influenza vaccines are widely used to prevent influenza disease in poultry. However, these vaccines have serious limitations, and in the event of a pandemic, they will not meet the needs of the poultry industry for vaccines. Due to the advancement of technology, researchers have been attracted to the development of recombinant influenza vaccines (Athmaram et al., 2011; Barone et al., 2023). These vaccines, which utilize biotechnology and molecular biology developments, present a viable substitute for conventional immunization techniques. Recombinant DNA technology is utilized to manufacture and deliver particular influenza viral antigens orally, thereby inducing systemic and mucosal immune responses in vaccinated animals (Wasilenko et al., 2010). It is worth mentioning that some influenza subtypes, such as H9N2, have become endemic in a vast geographical area of the Middle East (Nili & Asasi, 2003; Motamedi Nasab et al., 2023). It has been indicated that influenza viruses can evolve through point mutations and genetic reassortment, which can result in pathogenicity and host preference changes (Gong et al., 2021). Potentially, the H9N2 influenza subtype threatens public health and various researchers have mentioned it as the next global pandemic agent (Perdue & Swayne, 2005; Morens et al., 2023). Therefore, focusing on producing new and effective influenza vaccines is so important.

P. pastoris yeast has been recognized as a promising host for generating recombinant proteins and recombinant DNA technology has been employed to develop novel vaccines against avian influenza. It has been established that *P. pastoris* can be safely injected into mice and used as a safe vaccine-development delivery system (Becerril-García et al., 2022).

P. pastoris is an ideal host for influenza vaccine production that can overcome the drawbacks of inactive vaccines (Barone et al., 2023). In addition to having characteristics similar to mammalian cells, *P. pastoris* can be easily manipulated genetically, making the production of recombinant proteins in this yeast system economically viable (Wu et al., 2023). In addition, this yeast can rapidly express proteins and their translational and post-translational processing (Li et al., 2007). These factors have made this yeast a promising organism in producing eukaryotic proteins. Also, it is possible to achieve high cell density by using a bioreactor. Besides, *P. pastoris* has a special secretion system, so it secretes a very small amount of its intrinsic proteins into the culture medium; therefore, the cost of protein purification and subsequent processing is reduced. *P. pastoris* can form disulfide bonds and O- and N-linked glycosylation (Kuruti et al., 2020). This yeast does not cause hyperglycosylation of glycoproteins because it only adds short oligosaccharide chains to proteins. Recently, a lot of research has been done on this yeast to engineer its genome in a way that makes it more suitable for the production of recombinant proteins at high cell density (Tanaka et al., 2012; Kuruti et al., 2020; Zhang et al., 2020).

In this review, *P. pastoris* was illustrated as a suitable expression platform for creating recombinant antigens for the veterinary medicine and poultry industry. Some influenza vaccines produced by using this yeast system have been dramatically effective, could elicit high antibody titers and could protect animals from challenges with wild strains. Considering the benefits of *P. pastoris*, it is necessary to conduct more studies on developing universal recombinant influenza vaccines using this yeast.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research was supported by a research project, approved by the University of Tehran (Grant No.: 7825381).

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors wish to express their appreciation to everyone that assists us in this study and also would like to thank the research council of University of Tehran.

References

Abtin, A., Shoushtari, A., Pourbakhsh, S. A., Fallah Mehrabadi, M. H., & Pourtaghi, H. (2022). Two novel avian influenza virus subtypes isolated from domestic Ducks in North of Iran. *Archives of Razi Institute, 77*(2), 861–867. [PMID]

Abubakar, M. B., Aini, I., Omar, A. R., & Hair-Bejo, M. (2011). Cloning and expression of highly pathogenic avian influenza virus full-length nonstructural gene in Pichia pastoris. *Journal of Biomedicine & Biotechnology, 2011,* 414198. [DOI:10.1155/2011/414198] [PMID] [PMCID]

Alizadeh, E., Kheiri, M. T., Bashar, R., Tabatabaeian, M., Hosseini, S. M., & Mazaheri, V. (2009). Avian Influenza (H9N2) among poultry workers in Iran. *Iranian Journal of Microbiology, 1*(3), 3-6. [Link]

Alizadeh, J., Ranjbar, R., Kamali, M., Farhadi, N., Davari, A., & Sadeghifard, N. (2013). Cloning of Vibrio cholerae outer membrane protein W in Pichia pastoris. *Iranian Journal of Microbiology, 5*(3), 252-258. [PMID]

Asghari Baghkheirati, A., Sekhavati, M. H., Peighambari, S. M., Ghazvini, K., & Razmyar, J. (2023). Serological evaluation of H9-RBD-Pichia, a novel recombinant influenza vaccine, in BALB/c mice. *Iranian Journal of Veterinary Medicine.* [Link]

Athmaram, T. N., Saraswat, S., Santhosh, S. R., Singh, A. K., Suryanarayana, W. S., & Priya, R., et al. (2011). Yeast expressed recombinant Hemagglutinin protein of novel H1N1 elicits neutralising antibodies in rabbits and mice. *Virology Journal, 8,* 524. [PMID] [PMCID]

Azghandi, M., Tahmoorespur, M., & Sekhavati, M. H. (2022). [Comparison of antiviral effect of camel lactoferrin peptide (CLF36) and new generation drugs against hepatitis C virus (Persian)]. *Agricultural Biotechnology Journal, 14*(2), 21-44. [Link]

Barone, G. D., Emmerstorfer-Augustin, A., Biundo, A., Pisano, I., Coccetti, P., & Mapelli, V., et al. (2023). Industrial production of proteins with Pichia pastoris-Komagataella phaffii. *Biomolecules, 13*(3), 441. [PMID] [PMCID]

Becerril-García, M. Á., Flores-Maldonado, O. E., González, G. M., García-González, G., Hernández-Bello, R., & Palma-Nicolás, J. P. (2022). Safety profile of intravenous administration of live Pichia pastoris cells in mice. *FEMS Yeast Research, 22*(1), foac023. [PMID]

Cámara, E., Landes, N., Albiol, J., Gasser, B., Mattanovich, D., & Ferrer, P. (2017). Increased dosage of AOX1 promoter-regulated expression cassettes leads to transcription attenuation of the methanol metabolism in Pichia pastoris. *Scientific Reports, 7,* 44302. [PMID] [PMCID]

Daneshmand, A., Kermanshahi, H., Mohammed, J., Sekhavati, M. H., Javadmanesh, A., & Ahmadian, M., et al. (2022). Intestinal changes and immune responses during Clostridium perfringens-induced necrotic enteritis in broiler chickens. *Poultry Science, 101*(3), 101652. [DOI:10.1016/j.psj.2021.101652] [PMID] [PMCID]

de Sá Magalhães, S., & Keshavarz-Moore, E. (2021). Pichia pastoris (Komagataella phaffii) as a cost-effective tool for vaccine production for low-and middle-income countries (LMICs). *Bioengineering, 8*(9), 119. [PMID] [PMCID]

De, S., Mattanovich, D., Ferrer, P., & Gasser, B. (2021). Established tools and emerging trends for the production of recombinant proteins and metabolites in Pichia pastoris. *Essays in Biochemistry, 65*(2), 293-307. [DOI:10.1042/EBC20200138] [PMID]

Ebrahimi, S. M., Tebianian, M., Toghyani, H., Memarnejadian, A., & Attaran, H. R. (2010). Cloning, expression and purification of the influenza A (H9N2) virus M2e antigen and truncated Mycobacterium tuberculosis HSP70 as a fusion protein in Pichia pastoris. *Protein Expression and Purification, 70*(1), 7-12. [PMID]

Farsiani, H., Mosavat, A., Soleimanpour, S., Sadeghian, H., Akbari Eydgahi, M. R., & Ghazvini, K., et al. (2016). Fc-based delivery system enhances immunogenicity of a tuberculosis subunit vaccine candidate consisting of the ESAT-6: CFP-10 complex. *Molecular BioSystems, 12*(7), 2189-2201. [DOI:10.1039/c6mb00174b] [PMID]

França, R. C., Conceição, F. R., Mendonça, M., Haubert, L., Sabadin, G., & de Oliveira, P. D., et al. (2015). Pichia pastoris X-33 has probiotic properties with remarkable antibacterial activity against Salmonella Typhimurium. *Applied Microbiology and Biotechnology, 99*(19), 7953–7961. [DOI:10.1007/s00253-015-6696-9] [PMID]

Gaboardi, G. C., Alves, D., Gil de Los Santos, D., Xavier, E., Nunes, A. P., & Finger, P., et al. (2019). Influence of Pichia pastoris X-33 produced in industrial residues on productive performance, egg quality, immunity, and intestinal morphometry in quails. *Scientific Reports, 9*(1), 15372. [PMID] [PMCID]

Ghazvini, K., Neshani, A., Farsiani, H., Youssefi, M., & Keikha, M. (2021). Preparation and evaluation of antibacterial properties of Pexiganan, a Magainin analogue with Broadly-Spectrum Antimicrobial Activity. *Pakistan Journal of Medical & Health Sciences, 15*(6), 1778-1784. [Link]

Gholami, A., Shafiei-Jandaghi, N. Z., Ghavami, N., Tavakoli, F., Yavarian, J., & Mokhtari-Azad, T. (2022). Assessment of influenza A (H1N1, H3N2) oseltamivir resistance during 2017-2019 in Iran. *Iranian Journal of Microbiology, 14*(4), 545-553. [DOI:10.18502/ijm.v14i4.10241] [PMID] [PMCID]

Golgol, E., Mayahi, M., Boroomand, Z., & Shoshtari, A. (2023). Effect of vaccination on distribution and immune response of avian influenza virus H9N2 in Coturnix coturnix. *Archives of Razi Institute, 78*(6), 1746-1752. [DOI:10.32592/ARI.2023.78.6.1746] [PMID] [PMCID]

Gong, X., Hu, M., Chen, W., Yang, H., Wang, B., & Yue, J., et al. (2021). Reassortment network of influenza A virus. *Frontiers in Microbiology, 12,* 793500. [DOI:10.3389/fmicb.2021.793500] [PMID] [PMCID]

Hwang, J. S., Yamada, K., Honda, A., Nakade, K., & Ishihama, A. (2000). Expression of functional influenza virus RNA polymerase in the methylotrophic yeast Pichia pastoris. *Journal of Virology, 74*(9), 4074-4084. [DOI:10.1128/jvi.74.9.4074-4084.2000] [PMID] [PMCID]

Kebriaei, A., Derakhshan, M., Meshkat, Z., Eidgahi, M. R., Rezaee, S. A., & Farsiani, H., et al. (2016). Construction and immunogenicity of a new Fc-based subunit vaccine candidate against Mycobacterium tuberculosis. *Molecular Biology Reports, 43*(9), 911–922. [DOI:10.1007/s11033-016-4024-9] [PMID]

Kim, Y. H., Hong, K. J., Kim, H., & Nam, J. H. (2022). Influenza vaccines: Past, present, and future. *Reviews in Medical Virology, 32*(1), e2243. [DOI:10.1002/rmv.2243] [PMID] [PMCID]

Kingston, N. J., Snowden, J. S., Martyna, A., Shegdar, M., Grehan, K., & Tedcastle, A., et al. (2023). Production of antigenically stable enterovirus A71 virus-like particles in Pichia pastoris as a vaccine candidate. *The Journal of General Virology, 104*(6), 001867. [DOI:10.1099/jgv.0.001867] [PMID]

Kopera, E., Dwornyk, A., Kosson, P., Florys, K., Sączyńska, V., & Dębski, J., et al. (2014). Expression, purification and characterization of glycosylated influenza H5N1 hemagglutinin produced in Pichia pastoris. *Acta Biochimica Polonica, 61*(3), 597-602. [DOI:10.18388/abp.2014_1882] [PMID]

Kopera, E., Zdanowski, K., Uranowska, K., Kosson, P., Sączyńska, V., & Florys, K., et al. (2019). High-titre neutralizing antibodies to H1N1 influenza virus after mouse immunization with yeast expressed H1 antigen: A promising influenza vaccine candidate. *Journal of Immunology Research, 2019,* 2463731. [PMID] [PMCID]

Kulkarni, R. R., Gaghan, C., Gorrell, K., Sharif, S., & Taha-Abdelaziz, K. (2022). Probiotics as alternatives to antibiotics for the prevention and control of necrotic enteritis in chickens. *Pathogens, 11*(6), 692. [PMID] [PMCID]

Kuruti, K., Vittaladevaram, V., Urity, S. V., Palaniappan, P., & Bhaskar, R. U. (2020). Evolution of Pichia pastoris as a model organism for vaccines production in healthcare industry. *Gene Reports, 21,* 100937. [DOI:10.1016/j.genrep.2020.100937]

Li, J., Shi, L. W., Yu, B. W., Huang, L. R., Zhou, L. Y., & Shi, L., et al. (2023). Safety and immunogenicity of a pichia pastoris-expressed bivalent human papillomavirus (types 16 and 18) L1 virus-like particle vaccine in healthy Chinese women aged 9-45 years: A randomized, double-blind, placebo-controlled phase 1 clinical trial. *Vaccine, 41*(19), 3141-3149. [DOI:10.1016/j.vaccine.2023.04.009] [PMID]

Li, K., Gao, H., Gao, L., Qi, X., Gao, Y., & Qin, L., et al. (2012). Recombinant gp90 protein expressed in Pichia pastoris induces a protective immune response against reticuloendotheliosis virus in chickens. *Vaccine, 30*(13), 2273-2281. [DOI:10.1016/j.vaccine.2012.01.075] [PMID]

Li, P., Anumanthan, A., Gao, X. G., Ilangovan, K., Suzara, V. V., & Düzgüneş, N., et al. (2007). Expression of recombinant proteins in Pichia pastoris. *Applied Biochemistry and Biotechnology, 142*(2), 105–124. [DOI:10.1007/s12010-007-0003-x] [PMID]

Lin, Q., Yang, K., He, F., Jiang, J., Li, T., & Chen, Z., et al. (2016). Production of influenza virus HA1 harboring native-like epitopes by Pichia pastoris. *Applied Biochemistry and Biotechnology, 179*(7), 1275–1289. [DOI:10.1007/s12010-016-2064-1] [PMID]

Liu, B., Shi, P., Wang, T., Zhao, Y., Lu, S., & Li, X., et al. (2020). Recombinant H7 hemagglutinin expressed in glycoengineered Pichia pastoris forms nanoparticles that protect mice from challenge with H7N9 influenza virus. *Vaccine, 38*(50), 7938-7948. [DOI:10.1016/j.vaccine.2020.10.061] [PMID]

Maleknia, S., Ahmadi, H., & Norouzian, D. (2011). Immobilization of Pichia pastoris cells containing alcohol oxidase activity. I*ranian Journal of Microbiology, 3*(4), 210–215. [PMID]

Martinet, W., Saelens, X., Deroo, T., Neirynck, S., Contreras, R., & Min Jou, W., et al. (1997). Protection of mice against a lethal influenza challenge by immunization with yeast-derived recombinant influenza neuraminidase. *European Journal of Biochemistry, 247*(1), 332-338. [DOI:10.1111/j.1432-1033.1997.00332.x] [PMID]

Mayahi, M., Jolodar, A., Masaeli, S., Hamidinejat, H., Seyfi Abad Shapouri, M., & Moori Bakhtiari, N. (2016). Cloning and expression of Eimeria necatrix microneme5 gene in Escherichia coli. Iranian Journal of Veterinary Medicine, 10(3), 157-163. [DOI: 10.22059/ijvm.2016.58677]

Mirzaiee, K., Shoushtari, A., Bokaie, S., Fallah Mehrabadi, M. H., & Peighambari, S. M. (2020). Trend of changes in the titer of antibody against avian influenza virus H9N2 during raising period in vaccinated and unvaccinated broiler farms in Qazvin province, Iran: A cohort study. *Archives of Razi Institute, 75*(1), 9-16. [PMID]

Mirzaie, K., Shushtari, A., Bokaie, S., Fallah Mehrabadi, M., & Peighambari S. (2021). [Evaluation of H9N2 infection determinants in Qazvin broiler farms during 2016-17: A cohort study (Persian)]. *Iranian Journal of Epidemiology, 16*(4), 325-334. [Link]

Mohammadi, E., Pirkhezranian, Z., Dashty, S., Saedi, N., & Sekhavati, M. H. (2021). Design and computational analysis of a chimeric avian influenza antigen: A yeast-displayed, universal and cross-protective vaccine candidate. BioRxiv. [Link]

Mohammadzadeh, R., Karbalaei, M., Soleimanpour, S., Mosavat, A., Rezaee, S. A., & Ghazvini, K., et al. (2021). Practical methods for expression of recombinant protein in the Pichia pastoris system. *Current Protocols, 1*(6), e155. [DOI:10.1002/cpz1.155] [PMID]

Morens, D. M., Park, J., & Taubenberger, J. K. (2023). Many potential pathways to future pandemic influenza. *Science Translational Medicine, 15*(718), eadj2379. [DOI:10.1126/scitranslmed.adj2379] [PMID]

Moridi, K., Hemmaty, M., Eidgahi, M. R. A., Najafi, M. F., Zare, H., & Ghazvini, K., et al. (2020). Construction, cloning, and expression of Melittin antimicrobial peptide using Pichia pastoris expression system. *Gene Reports, 21,* 100900. [DOI:10.1016/j.genrep.2020.100900]

Mosavat, A., Soleimanpour, S., Farsiani, H., Sadeghian, H., Ghazvini, K., & Sankian, M., et al. (2016). Fused Mycobacterium tuberculosis multi-stage immunogens with an Fc-delivery system as a promising approach for the development of a tuberculosis vaccine. *Infection, Genetics and Evolution, 39,* 163-172. [DOI:10.1016/j.meegid.2016.01.027] [PMID]

Motamedi Nasab, S. I., Pourbakhsh, S. A., & Haghbin Nazarpak, H. (2023). Evaluation of inactivated vaccine's Antibody response to different H9N2 Vaccination programs with Hemagglutination Inhibition (HI) assay. *Journal of Poultry Sciences and Avian Diseases, 1*(3), 51-58. [DOI:10.61838/kman.jpsad.1.3.5]

Mu, X., Hu, K., Shen, M., Kong, N., Fu, C., & Yan, W., et al. (2016). Protection against influenza A virus by vaccination with a recombinant fusion protein linking influenza M2e to human serum albumin (HSA). *Journal of Virological Methods, 228,* 84-90. [DOI:10.1016/j.jviromet.2015.11.014] [PMID]

Mukhopadhyay, E., Brod, F., Angell-Manning, P., Green, N., Tarrant, R. D., & Detmers, F. J., et al. (2022). Production of a high purity, C-tagged hepatitis B surface antigen fusion protein VLP vaccine for malaria expressed in Pichia pastoris under cGMP conditions. *Biotechnology and Bioengineering, 119*(10), 2784-2793. [DOI: 10.1002/bit.28181] [PMID] [PMCID]

Murugan, S., Ponsekaran, S., Kannivel, L., Mangamoori, L. N., Chandran, D., & Villuppanoor Alwar, S., et al. (2013). Recombinant haemagglutinin protein of highly pathogenic avian influenza A (H5N1) virus expressed in Pichia pastoris elicits a neutralizing antibody response in mice. *Journal of Virological Methods, 187*(1), 20-25. [DOI:10.1016/j.jviromet.2012.07.026] [PMID]

Neshani, A., Eidgahi, M. R. A., Zare, H., & Ghazvini, K. (2018). Extended-Spectrum antimicrobial activity of the Low cost produced Tilapia Piscidin 4 (TP4) marine antimicrobial peptide. *Journal of Research in Medical and Dental Science, 6*(5), 327-334. [Link]

Neshani, A., Tanhaeian, A., Zare, H., Eidgahi, M. R. A., & Ghazvini, K. (2019). Preparation and evaluation of a new biopesticide solution candidate for plant disease control using pexiganan gene and Pichia pastoris expression system. *Gene Reports, 17*, 100509. [DOI:10.1016/j.genrep.2019.100509]

Nguyen, T. Q., Van, T. T. H., Lin, Y. C., Van, T. N. N., Bui, K. C., & Le, Q. G., et al. (2014). A potential protein-based vaccine for influenza H5N1 from the recombinant HA1 domain of avian influenza A/H5N1 expressed in Pichia pastoris. *Future Virology, 9*(12), 1019-1031. [Link]

Nili, H., & Asasi, K. (2003). Avian influenza (H9N2) outbreak in Iran. *Avian Diseases, 47*(3 Suppl), 828–831. [DOI:10.1637/0005-2086-47.s3.828] [PMID]

Norouzian, H., Bashashati, M., & Vasfimarandi, M. (2014). Phylogenetic analysis of neuraminidase gene of H9N2 avian influenza viruses isolated from chicken in Iran during 2010-2011. *Iranian Journal of Microbiology, 6*(2), 91–97. [PMID]

Noseda, D. G., D'Alessio, C., Santos, J., Idrovo-Hidalgo, T., Pignataro, F., & Wetzler, D. E., et al. (2023). Development of a cost-effective process for the heterologous production of SARS-CoV-2 spike receptor binding domain using Pichia pastoris in stirred-tank bioreactor. *Fermentation, 9*(6), 497. [DOI:10.3390/fermentation9060497]

Nouri Gharajalar, S., Ahmadi, M., Shahabi, S., & Hosseini, B. (2016). Use of immunogenic moiety of Pseudomonas aeruginosa exotoxin A as a DNA vaccine in experimentally contaminated mice. *Iranian Journal of Veterinary Medicine, 10*(2), 105-112. [DOI:10.22059/IJVM.2016.57896]

Peighambari, S. M., Yazdani, A., Taheri, H., & Shahcheraghi, F. (2023). Plasmid profile and enterobacterial repetitive intergenic consensus-PCR characterization of salmonella infantis isolates recovered from poultry sources. *Iranian Journal of Veterinary Medicine, 17*(1), 27-36. [DOI:10.32598/IJVM.17.1.1005073]

Perdue, M. L., & Swayne, D. E. (2005). Public health risk from avian influenza viruses. *Avian Diseases, 49*(3), 317-327. [DOI:10.1637/7390-060305R.1] [PMID]

Pietrzak, M., Macioła, A., Zdanowski, K., Protas-Klukowska, A. M., Olszewska, M., & Śmietanka, K., et al. (2016). An avian influenza H5N1 virus vaccine candidate based on the extracellular domain produced in yeast system as subviral particles protects chickens from lethal challenge. *Antiviral Research, 133*, 242-249. [DOI:10.1016/j.antiviral.2016.08.001] [PMID]

Pratanaphon, R., Channoi, P., & Suphawilai, C. (2018). Cloning and Expression of HA2 gene of Avian Influenza A (H5N1 HA2) Virus in Pichia pastoris. *Food and Applied Bioscience Journal, 6*(2), 106-116. [DOI:10.14456/fabj.2018.10]

Qian, X. U. E., Wenge, M. A., Kayizha, S., Ping, W. A. N. G., Tao, H. A. N., & Shukui, M. I. A. O., et al. (2021). Avian influenza virus-like particles expressed and assembled by multi-copy recombinant pichia pastoris. *Xinjiang Agricultural Sciences, 58*(11), 2148. [DOI: 10.6048/j.issn.1001-4330.2021.11.022]

Ravansalar, H., Tadayon, K., & Ghazvini, K. (2016). Molecular typing methods used in studies of Mycobacterium tuberculosis in Iran: A systematic review. *Iranian Journal of Microbiology, 8*(5), 338-346. [PMID]

Rungrojcharoenkit, K., Sunintaboon, P., Ellison, D., Macareo, L., Midoeng, P., & Chaisuwirat, P., et al. (2020). Development of an adjuvanted nanoparticle vaccine against influenza virus, an in vitro study. *PLoS One, 15*(8), e0237218. [DOI:10.1371/journal.pone.0237218] [PMID] [PMCID]

Saelens, X., Vanlandschoot, P., Martinet, W., Maras, M., Neirynck, S., & Contreras, R., et al. (1999). Protection of mice against a lethal influenza virus challenge after immunization with yeast-derived secreted influenza virus hemagglutinin. *European Journal of Biochemistry, 260*(1), 166-175. [DOI:10.1046/j.1432-1327.1999.00150.x] [PMID]

Sahebnazar, A., Tahmoorepur, M., & Sekhavati, M. H. (2021). Molecular docking CLF36 peptide against avian influenza virus subtype H5N8 antigenes. *Veterinary Research & Biological Products, 34*(4), 54-65. [DOI:10.22092/VJ.2020.351493.1754]

Salamatian, I., Moshaverinia, A., Razmyar, J., & Ghaemi, M. (2020). In vitro Acquisition and Retention of Low-Pathogenic Avian Influenza H9N2 by Musca domestica (Diptera: Muscidae). *Journal of Medical Entomology, 57*(2), 563–567. [DOI:10.1093/jme/tjz175] [PMID] [PMCID]

SGil de Los Santos, D., Gil de Los Santos, J. R., Gil-Turnes, C., Gaboardi, G., Fernandes Silva, L., &França, R., et al. (2018). Probiotic effect of Pichia pastoris X-33 produced in parboiled rice effluent and YPD medium on broiler chickens. *Plos One, 13*(2), e0192904. [PMID] [PMCID]

Seyedtaghiya, M. H., Fasaei, B. N., & Peighambari, S. M. (2021). Antimicrobial and antibiofilm effects of Satureja hortensis essential oil against Escherichia coli and Salmonella isolated from poultry. *Iranian Journal of Microbiology, 13*(1), 74–80. [DOI:10.18502/ijm.v13i1.5495] [PMID] [PMCID]

Shehata, A. A., Fiebig, P., Sultan, H., Hafez, M., & Liebert, U. G. (2012). Development of a recombinant ELISA using yeast (Pichia pastoris)-expressed polypeptides for detection of antibodies against avian influenza A subtype H5. *Journal of Virological Methods, 180*(1-2), 18-25. [DOI:10.1016/j.jviromet.2011.12.004] [PMID]

Shirdast, H., Ebrahimzadeh, F., Taromchi, A. H., Mortazavi, Y., Esmaeilzadeh, A., & Sekhavati, M. H., et al. (2021). Recombinant Lactococcus Lactis Displaying Omp31 antigen of Brucella melitensis can induce an immunogenic response in BALB/c Mice. *Probiotics and Antimicrobial Proteins, 13*(1), 80–89. [DOI:10.1007/s12602-020-09684-1] [PMID]

Singh, A., & Narang, A. (2020). The Mut+ strain of Komagataella phaffii (Pichia pastoris) expresses P AOX1 5 and 10 times faster than Mut s and Mut− strains: Evidence that formaldehyde or/and formate are true inducers of P AOX1. *Applied Microbiology and Biotechnology, 104*(18), 7801–7814. [DOI:10.1007/s00253-020-10793-8] [PMID]

Soleimanpour, S., Farsiani, H., Mosavat, A., Ghazvini, K., Eydgahi, M. R., & Sankian, M., et al. (2015). APC targeting enhances immunogenicity of a novel multistage Fc-fusion tuberculosis vaccine in mice. *Applied Microbiology and Biotechnology, 99,* 10467-10480. [DOI: 10.1007/s00253-015-6952-z] [PMID]

Stachyra, A., Pietrzak, M., Macioła, A., Protasiuk, A., Olszewska, M., & Śmietanka, K., et al. (2017). A prime/boost vaccination with HA DNA and Pichia-produced HA protein elicits a strong humoral response in chickens against H5N1. *Virus Research, 232,* 41-47. [DOI:10.1016/j.virusres.2017.01.025] [PMID]

Stubbs, A. C., Martin, K. S., Coeshott, C., Skaates, S. V., Kuritzkes, D. R., & Bellgrau, D., et al. (2001). Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. *Nature Medicine, 7*(5), 625-629. [DOI:10.1038/87974] [PMID]

Subathra, M., Santhakumar, P., Satyam Naidu, S., Lakshmi Narasu, M., Senthilkumar, T. M., & Lal, S. K. (2014a). Expression of avian influenza virus (H5N1) hemagglutinin and matrix protein 1 in Pichia pastoris and evaluation of their immunogenicity in mice. *Applied Biochemistry and Biotechnology, 172*(7), 3635–3645. [DOI:10.1007/s12010-014-0771-z] [PMID]

Subathra, M., Santhakumar, P., Narasu, M. L., Beevi, S. S., & Lal, S. K. (2014b). Evaluation of antibody response in mice against avian influenza A (H5N1) strain neuraminidase expressed in yeast Pichia pastoris. *Journal of Biosciences, 39*(3), 443–451. [DOI:10.1007/s12038-014-9422-3] [PMID]

Sulfianti, A., Yasmon, A., Bela, B., & Ibirahim, F. (2015). Cloning and Expression of HA1 Gene of H1N1 Influenza Virus 2009 Pandemic (H1n1pdm09) Indonesia Strain in the Pichia Pastoris Expression System for the Development of Influenza Vaccine. *Microbiology Indonesia, 9*(2), 7. [DOI:10.5454/mi.9.2.7]

Taghavian, O., Spiegel, H., Hauck, R., Hafez, H. M., Fischer, R., & Schillberg, S. (2013). Protective oral vaccination against infectious bursal disease virus using the major viral antigenic protein VP2 produced in Pichia pastoris. *PLoS One, 8*(12), e83210. [DOI:10.1371/journal.pone.0083210] [PMID] [PMCID]

Taghizadeh, M., & Dabaghian, M. (2022). Nasal Administration of M2e/CpG-ODN Encapsulated in N-Trimethyl Chitosan (TMC) Significantly Increases Specific Immune Responses in a Mouse Model. *Archives of Razi Institute, 77*(6), 2259–2268. [DOI:10.22092/ARI.2022.360447.2583] [PMID]

Tanaka, T., Yamada, R., Ogino, C., & Kondo, A. (2012). Recent developments in yeast cell surface display toward extended applications in biotechnology. *Applied Microbiology and Biotechnology, 95*(3), 577–591. [DOI:10.1007/s00253-012-4175-0] [PMID]

Wang, S. C., Liao, H. Y., Zhang, J. Y., Cheng, T. R., & Wong, C. H. (2019). Development of a universal influenza vaccine using hemagglutinin stem protein produced from Pichia pastoris. *Virology, 526,* 125-137. [DOI:10.1016/j.virol.2018.10.005] [PMID]

Wasilenko, J. L., Sarmento, L., Spatz, S., & Pantin-Jackwood, M. (2010). Cell surface display of highly pathogenic avian influenza virus hemagglutinin on the surface of Pichia pastoris cells using α-agglutinin for production of oral vaccines. *Biotechnology Progress, 26*(2), 542-547. [DOI:10.1002/btpr.343] [PMID]

Wollborn, D., Munkler, L. P., Horstmann, R., Germer, A., Blank, L. M., & Büchs, J. (2022). Predicting high recombinant protein producer strains of Pichia pastoris MutS using the oxygen transfer rate as an indicator of metabolic burden. *Scientific Reports, 12*(1), 11225. [DOI:10.1038/s41598-022-15086-w] [PMID] [PMCID]

Wu, S., & Letchworth, G. J. (2004). High efficiency transformation by electroporation of Pichia pastoris pretreated with lithium acetate and dithiothreitol. *Biotechniques, 36*(1), 152-154. [DOI:10.2144/04361DD02] [PMID]

Wu, X., Cai, P., Yao, L., & Zhou, Y. J. (2023). Genetic tools for metabolic engineering of Pichia pastoris. *Engineering Microbiology, 3*(4), 100094. [DOI:10.1016/j.engmic.2023.100094]

Xu, H., Wang, T., Sun, P., Hou, X., Gong, X., & Zhang, B., et al. (2023). A bivalent subunit vaccine efficiently produced in Pichia pastoris against SARS-CoV-2 and emerging variants. *Frontiers in Microbiology, 13,* 1093080. [DOI:10.3389/fmicb.2022.1093080] [PMID] [PMCID]

Yang, Z. J., Wang, C. Y., Lee, L. H., Chuang, K. P., Lien, Y. Y., Yin, H. S., et al. (2010). Development of ELISA kits for antibodies against avian reovirus using the σC and σB proteins expressed in the methyltropic yeast Pichia pastoris. *Journal of Virological Methods, 163*(2), 169-174. [DOI:10.1016/j.jviromet.2009.07.009] [PMID]

Yang, Y. L., Chang, S. H., Gong, X., Wu, J., & Liu, B. (2012). Expression, purification and characterization of low-glycosylation influenza neuraminidase in α-1, 6-mannosyltransferase defective Pichia pastoris. *Molecular Biology Reports, 39*(2), 857–864. [DOI:10.1007/s11033-011-0809-z] [PMID]

Yongkiettrakul, S., Boonyapakron, K., Jongkaewwattana, A., Wanitchang, A., Leartsakulpanich, U., & Chitnumsub, P., et al. (2009). Avian influenza A/H5N1 neuraminidase expressed in yeast with a functional head domain. *Journal of Virological Methods, 156*(1-2), 44-51. [DOI:10.1016/j.jviromet.2008.10.025] [PMID] [PMCID]

Yousefi, S., Tahmoorespur, M., & Sekhavati, M. H. (2016). Cloning, expression and molecular analysis of Iranian Brucella melitensis Omp25 gene for designing a subunit vaccine. *Research in Pharmaceutical Sciences, 11*(5), 412-418. [DOI:10.4103/1735-5362.192493] [PMID] [PMCID]

Zhang, C., Ma, Y., Miao, H., Tang, X., Xu, B., & Wu, Q., et al. (2020). Transcriptomic analysis of Pichia pastoris (Komagataella phaffii) GS115 during heterologous protein production using a high-cell-density fed-batch cultivation strategy. *Frontiers in Microbiology, 11,* 463. [DOI:10.3389/fmicb.2020.00463] [PMID] [PMCID]

Zhang, J., Chen, P., Sun, H., Liu, Q., Wang, L., & Wang, T., et al. (2014). Pichia pastoris expressed EtMic2 protein as a potential vaccine against chicken coccidiosis. *Veterinary Parasitology, 205*(1-2), 62-69. [DOI:10.1016/j.vetpar.2014.06.029] [PMID]

Zhang, Y., Yang, S., Dai, X., Liu, L., Jiang, X., & Shao, M., et al. (2015). Protective immunity induced by the vaccination of recombinant Proteus mirabilis OmpA expressed in Pichia pastoris. *Protein Expression and Purification, 105,* 33-38. [DOI:10.1016/j.pep.2014.10.001] [PMID]

Zhao, F., Wang, Y., Chen, L., Zhang, X., Ducatez, M., & He, J., et al. (2021). Critical influenza-like illness in a nine-year-old associated with a poultry-origin H9N2 avian influenza virus: Risk assessment and zoonotic potential. *Frontiers in Virology, 1,* 727163. [Link]

مقاله مروری

پیکیا پاستوریس یک میزبان ایدئآل برای تولید واکسن‌های نوترکیب آنفلوانزا

امیر اصغری باغخیراتی[۱]، محمد هادی سخاوتی[۲]، سید مصطفی پیغمبری[۱]، کیارش قزوینی[۳]، *جمشید رزم یار[۱]

۱. گروه بهداشت و بیماری های پرندگان، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران.
۲. گروه علوم دامی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران.
۳. گروه میکروبیولوژی و ویروس شناسی، دانشکده پزشکی، دانشگاه علوم پزشکی مشهد، مشهد، ایران.

Use your device to scan and read the article online

How to Cite This Article Asghari Baghkheirati, A., Sekhavati, M. H., Peighambari, S. M., Ghazvini, K., & Razmyar, J. (2024). *Pichia pastoris* an Ideal Host for the Production of Recombinant Influenza Vaccines. *Iranian Journal of Veterinary Medicine, 18*(4), 465-478. http://dx.doi.org/10.32598/ijvm.18.4.1005523

http://dx.doi.org/10.32598/ijvm.18.4.1005523

چکیده

پیکیا پاستوریس یک مخمر متیلوتروف با ویژگی‌های قابل توجهی مانند نداشتن اندوتوکسین، تولید مقادیر بالای پروتئین نوترکیب، انجام تغییرات پس از ترجمه و غیره است. ویروس آنفلوانزای A، یکی از اعضای خانواده اورتومیکسوویریده است که عامل آنفلوانزای پرندگان می‌باشد. سه تحت تیپ H5، H7 و H9 ویروس آنفلوانزای پرندگان ازنظر تجاری و فیزیولوژیکی در صنعت طیور دارای اهمیت هستند. برخی از محققان، آنفلوانزای را بیماری همه‌گیر بعدی می‌دانند. امروزه توجه محققان به تولید واکسن‌های نوترکیب جدید و مؤثر به‌ویژه در صنعت طیور معطوف شده است. باتوجه‌به مزایای مخمر پیکیا پاستوریس می‌توان از آن به‌عنوان یک سیستم بیانی ایدئآل برای تولید واکسن‌های زیر واحد استفاده کرد. اگرچه مطالعات متعددی در این زمینه انجام شده است، اما مطالعه مروری جامعی درمورد استفاده از پیکیا پاستوریس برای تولید واکسن‌های نوترکیب آنفلوانزای وجود ندارد. در این مطالعه مروری، سویه‌ها، فنوتیپ‌ها و مزایای مختلف این مخمر توضیح داده شد و سپس درمورد تولید واکسن‌های نوترکیب آنفلوانزای با استفاده از این سیستم بیانی به‌طور خاص بحث شده است.

کلیدواژه‌ها: آنفلوانزای، پیکیا پاستوریس، نوترکیب، واکسن، دامپزشکی

تاریخ دریافت: ۰۴ خرداد ۱۴۰۲
تاریخ پذیرش: ۱۹ تیر ۱۴۰۲
تاریخ انتشار: ۱۰ مهر ۱۴۰۳

* **نویسنده مسئول:**
دکترجمشید رزم یار
نشانی: تهران، دانشگاه تهران، دانشکده دامپزشکی،گروه بهداشت و بیماری‌های پرندگان.
تلفن: ۶۱۱۱۷۰۰۰ (۲۱) ۹۸+
رایانامه: razmyar@ut.ac.ir