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

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Abstract

Pichia pastoris is a methylotrophic yeast with remarkable characteristics such as not having endotoxin, producing high amounts of recombinant protein, performing post-translational modifications, etc. 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 the production of 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. In this review, different strains, phenotypes, and advantages of this yeast are explained, and then the production of recombinant influenza vaccines using this expression system is specifically discussed.

Keywords: Influenza, *Pichia pastoris*, recombinant, vaccine, veterinary medicine.

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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

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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 to be the next pandemic disease (Morens *et al*., 2023). Approximately between 250,000 and 500,000 individuals die from influenza virus infections worldwide annually (Norouzian *et al*., 2014; Perdue and Swayne, 2005; Kim *et al*., 2021).

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.

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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 *E. 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, etc (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 and Dabaghian, 2022). Besides, new subunit vaccines have been made in medicine against SARS-CoV-2, enterovirus, papillomavirus, malaria, etc using *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 and Keshavarz-Moore, 2021). The objective of this review study is to describe *P. pastoris* as one of the most efficient expression systems for developing recombinant vaccines for the poultry industry, with a particular focus 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 subsequently the use of methanol, this yeast can be classified into three phenotype categories (Maleknia *et al*., 2011; Singh and 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 both *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 and 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 use methanol quickly. Since these strains use methanol slowly, more complex proteins will have time to

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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 and Narang, 2020).

The advantages of using *P. pastoris*

There are several reasons for using this yeast as an expression system, as 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 by using culture medium containing yeast extract, peptone, and dextrose (Kuruti *et al*., 2020).

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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

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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 *Saccharomyces 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 X-33 strain can survive in food at an appropriate

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concentration for at least two months. *Salmonella*, *Clostridium*, and *E. coli* are among the most important bacterial pathogens in the poultry industry that have caused significant economic losses (Seyedtaghiya *et al*., 2021; Daneshmand *et al*., 2022; Peighambari *et al*., 2023). *P. pastoris* can be used as a probiotic and antibiotic alternative to prevent and control these pathogens. This yeast's 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 IBV, ND, and 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 (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,

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expressed recombinant proteins and yeast cell wall components will be more immunogenic when administered (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 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 and Letchworth, 2004; Yongkiettrakul *et al*., 2009; Sulfianti *et al*., 2015; Pratanaphon *et al*., 2018). Furthermore, pre-treating yeast cells with lithium acetate and dithiothreitol has been shown to boost transformation efficiency significantly by 150-fold (Wu and Letchworth, 2004).

P. pastoris vectors

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There are two types of 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 used to express recombinant proteins in Pichia 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) have been 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). Some scientists delivered these proteins

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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 and 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 percent of the chickens injected with H5DHΔ had high titers of neutralizing antibodies in the HI assay. Interestingly, all the vaccinated chickens survived the challenge with H5N1, and no clinical symptoms were observed, but the control group chickens died on the 4th day after the challenge. This study shows that using the yeast system in the production of recombinant proteins as a subunit vaccine can be effective and protect chickens against lethal challenges. In research

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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 *Bgl*II 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 (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 correct production of hemagglutinin protein by the yeast. 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

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study of Subathra *et al*. (2014a) the sequence of *M1* and *HA* genes were 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 vaccines production, with and without in vivo test, were illustrated in table 1 and 2, respectively.

P. pastoris usage in the production of other recombinant vaccines

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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 infectious bursal disease 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).

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Discussion

Influenza is one of the most crucial diseases that has resulted in uncompensated losses to the poultry industry worldwide (Nili and 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 take advantage of developments in biotechnology and molecular biology, 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 and 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

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changes (Gong *et al*., 2021). Potentially, the H9N2 influenza subtype is a threat to public health, and various researchers have mentioned it as the next global pandemic agent (Perdue and 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 for developing 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, and this makes the production of recombinant proteins in this yeast system economically viable (Wu *et al*., 2023). In addition, this yeast has a high ability to rapidly express proteins as well as their translational and post-translational processing (Li *et al*., 2007). These factors have made this yeast a very promising organism in the production of 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

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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* is able to form disulfide bonds as well as 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.

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Tables

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

Influenza subtype	Antigen	*P. pastoris* stains	Vector	In vivo test	adjuvant	Result	Ref.

H3N2 (A/Victoria/3/75)	NA	GS115	pPIC 9	BALB/c mice, SC,3 times	First 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	pPIC 9	BALB/c mice, SC,3 times	First 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	pPIC Zα	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	pPIC 9K	Chicken Oral gavage	None	Production of virus neutralizing antibodies.	(Wasilenko *et al*., 2010)

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H5N1 (A/swan/Poland/ 305-135V08/2006)	HA	SMD 1168 and KM 71	pPIC ZαC	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	pPIC 9K	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	pPIC ZαC	Chicken, SC, twice	Aluminium hydroxide	Protection from lethal challenge.	(Pietrzak *et al*., 2016)
H5N1 (A/swan/Poland/ 305-135V08/2006)	HA	KM 71	pPIC ZαC	Chicken, SC, twice.	Alhydrogel	Eliciting a strong humoral response	(Stachyra *et al*., 2017)
H5N1	HA	SMD116	pPIC	Chicken,	Freund's adjuvant	HI values of up	(Nguyen *et al*.,

Uncorrected Proof

(A/Hatay/2004)		8	9	SC, twice.		to 7 log2.	2014)
H5N1 (A/Hatay/2004)	HA	P. pastoris	pPIC ZαC	Mice, SC, 3 times,	2% Aluminium hydroxide	A maximum of 280 HI titer.	(Murugan *et al*., 2013)
H5N1 (A/Hatay/2004)	HA & M1	GS115	pPIC ZαC	Swiss albino mice	2% Aluminium hydroxide	HA titer of 1:32	(Subathra *et al*., 2014a)
H5N1 (A/Hatay/2004)	NA	GS115	pPIC ZαC	Mice, SC, 3 times,	2% Aluminium hydroxide	Significant immune response against rNA. (ELISA).	(Subathra *et al*., 2014b)
H1N1 (A/newCaledonia/20/99)	NA (low-glycosylated NA)	GS115	pPIC 9	BALB/c mice, IP, 3 times.	Aluminium hydroxide	High antibody titer (1:4,900)	(Yang *et al*., 2012)
H1N1 (A/Brisbane/59/2007)	HA	KM71H	pPIC ZαA	BALB/c mice, IM, 3 times,	aluminium hydroxide	Broad protection in mice.	(Wang *et al*., 2019)

Uncorrected Proof

H1N1 (A/H1N1/Gdansk/036/2009)	HA	KM 71	pPIC ZαA	BALB/c mice, SC, 3 times,	Alhydrogel (aluminium hydroxide)	HI titres as high as 1 : 2048	(Kopera *et al*., 2019)
H1N1 (A/California/04/2009)	HA	GS115	pPIC 9K	Rabbits and mice, IM, twice.	First injection: Freund's complete adjuvant (FCA). Booster: Freund's Incomplete adjuvant (FIA)	Mean HI titres of 1:32	(Athmaram *et al*., 2011)
H7N9 (A/Hangzhou/1/2013)	HA	P. pastoris	pPIC ZαA	BALB/c mice, IM, twice.	Aluminium hydroxide	High antibody titer & complete protection	(Liu *et al*., 2020)

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

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Influenza subtype	Antigen	*P. pastoris* strains	Vector	Methanol concentraton (V/V)	Ref.
H1N1 (A/Jakarta/271/2011)	HA	GS115	pPICZα-A	1 %	(Sulfianti *et al*., 2015)
H5N1 .1 A/Thailand/3(SP-83)/2004)	HA2	GS115	pPICZα-A	1% every 12h	(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/07/200	HA2 and NP	*P. pastoris*	pPICZαB	1 % for 96h	(Rungrojcharoenkit *et*

Uncorrected Proof

9)					*al*., 2020)
H1N1 (A/PR/8)	PB1-PB2-PA	KM71	pPicZaA & pPic9	0.5 % every 24h for 96h	(Hwang *et al*., 2000)

Figure legends

Figure 1. Advantages of using *P. pastoris* in recombinant expression of influenza antigens according to the *in vitro* studies. The trimeric hemagglutinin antigen, the most important antigen in stimulating the immune system, was shown as a sample.

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

Uncorrected Proof

Figures

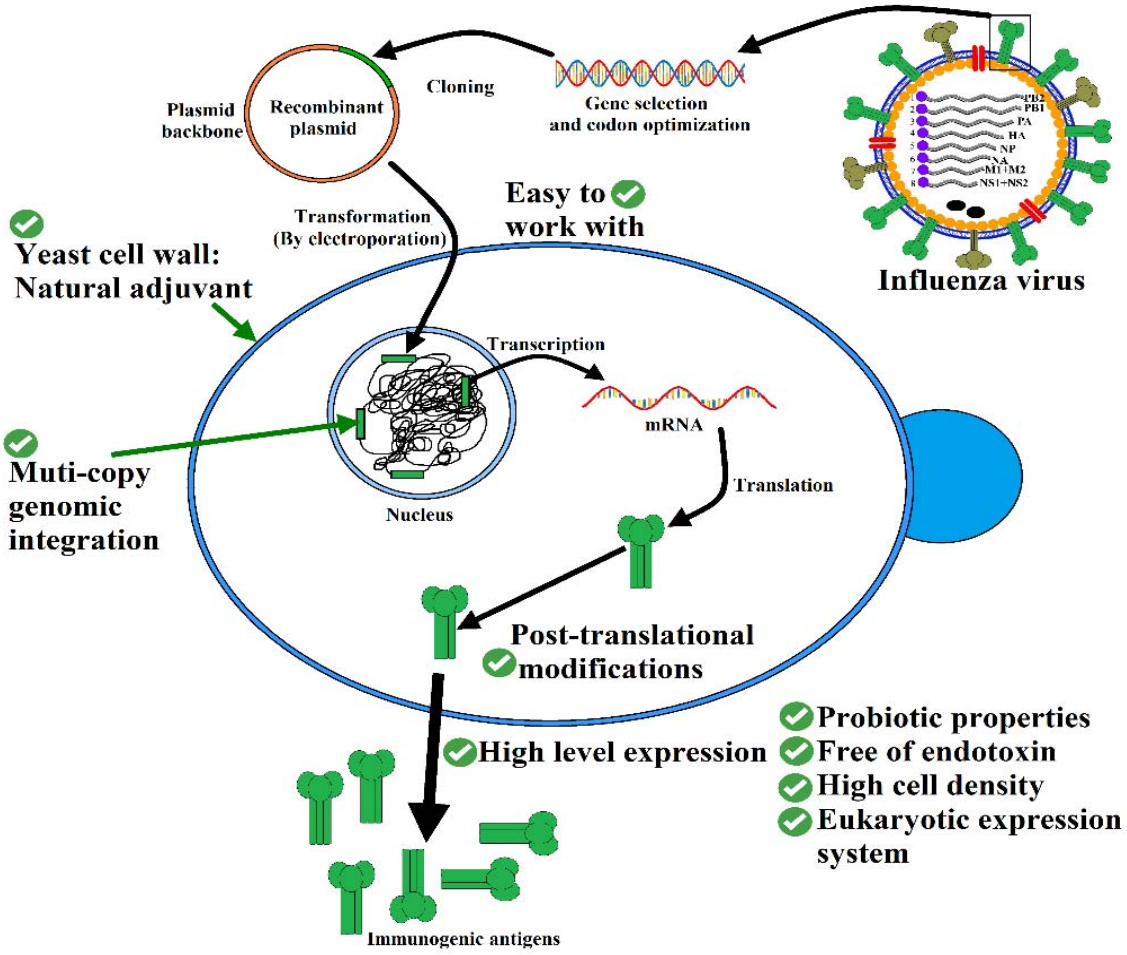

Figure 1.

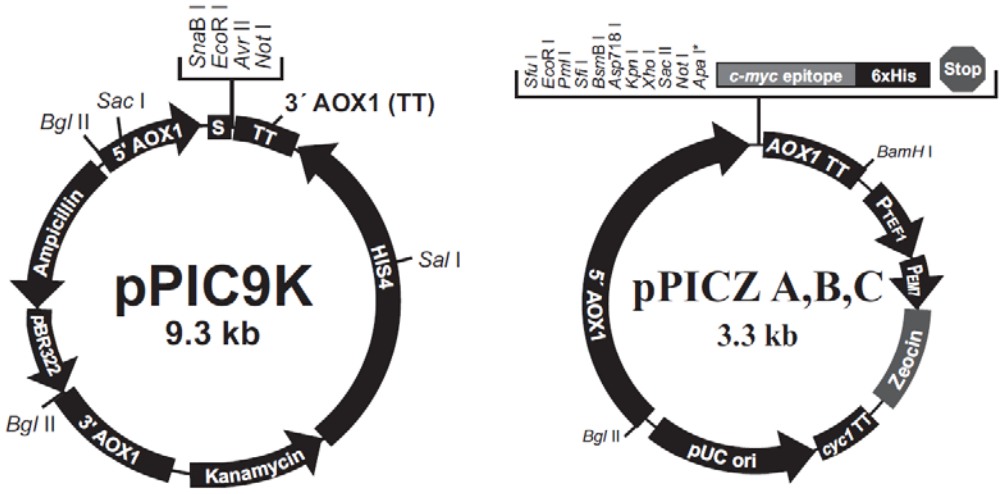
SnaB I
EcoR I
Avr II
Not I
3´ AOX1 (TT)
Sac I
Bgl II
5' AOX1
S
TT
Ampicillin
pPIC9K
9.3 kb
HIS4
Sal I
pBR322
Bgl II
3' AOX1
Kanamycin
Sfu I
EcoR I
Pml I
Sfi I
BsmB I
Asp718 I
Kpn I
Xho I
Sac II
Not I
Ape I*
c-myc epitope
6xHis
Stop
AOX1 TT
BamH I
PTEF1
pAM
Zeocin
cyc1 TT
pUC ori
Bgl II
5' AOX1
pPICZ A,B,C
3.3 kb

Figure 2.

پیکیا پاستوریس، یک میزبان ایده آل برای تولید واکسن های نوترکیب آنفلوانزا

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Corrected Pro

چکیده:

پیکیا پاستوریس یک مخمر متیلوتروف با ویژگی های قابل توجهی مانند نداشتن اندوتوکسین، تولید مقادیر بالای پروتئین نوترکیب، انجام تغییرات پس از ترجمه و غیره است. ویروس آنفولانزای A، یکی از اعضای خانواده اورتومیکسوویریده است که عامل آنفولانزای پرندگان می‌باشد. سه تحت تیپ H5، H7 و H9 ویروس آنفلوانزای پرندگان از نظر تجاری و فیزیولوژیکی در صنعت طیور دارای اهمیت هستند. برخی از محققان، آنفولانزا را بیماری همه گیر بعدی می دانند. امروزه توجه محققان به تولید واکسن های نوترکیب جدید و موثر به ویژه در صنعت طیور معطوف شده است. با توجه به مزایای مخمر پیکیا پاستوریس می توان از آن به عنوان یک سیستم بیانی ایده آل برای تولید واکسن های زیر واحد استفاده کرد. اگرچه مطالعات متعددی در این زمینه انجام شده است، اما مطالعه مروری جامعی در مورد استفاده از پیکیا پاستوریس برای تولید واکسن‌های نوترکیب آنفلوانزا وجود ندارد. در این مطالعه مروری، سویه‌ها، فنوتیپ‌ها و مزایای مختلف این مخمر توضیح داده شده و سپس تولید واکسن‌های نوترکیب آنفولانزا با استفاده از این سیستم بیانی به طور خاص مورد بحث قرار گرفته است.

کلیدواژه‌ها: آنفلوانزا، پیکیا پاستوریس، نوترکیب، واکسن، دامپزشکی.