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Review

Animal vaccines based on orally presented yeast recombinants

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ABSTRACT

In veterinary vaccinology, the oral route of administration is an attractive alternative compared to the commonly used parenteral route. Yeasts have a number of properties that make them potential live delivery systems for oral vaccination purposes such as their high expression levels, their GRAS status, adjuvant properties, and post-translational modification possibilities. Consequently, yeasts have been employed for the expression of heterologous genes and for the production of therapeutic proteins. Yeast-based vaccines are reviewed with regard to their ability to express and produce antigens from pathogens for veterinary use. Many of these vaccines have been shown to elicit protective immune responses following oral immunization in animals. Ultimately, yeast-based oral vaccines may offer a potential opportunity for the development of novel ideal vaccines in veterinary medicine.

Contents

1. Introduction

Infectious diseases cause animal deaths, economic losses, and public health issues. Veterinary vaccinology is one route toward reducing the problems associated with such diseases, and these vaccines have already reduced the consumption of veterinary drugs such as antibiotics, thus resulting in reduced environmental consequences, side effects, and residues in food animal products [1]. The vaccination of animals is implicated in a wider range of objectives such as in the provision of cost-effective systems to control infectious diseases in animals, animal welfare improvements, and in decreasing the cost of production in food animals [2]. Alongside animal health considerations, veterinary vaccinology is directly related to enhancing public health.

Most current licensed veterinary vaccines have been developed as killed or live modified vaccines for parenteral immunization [2]. These conventional vaccines require adjuvants and multiple administrations for the induction of sufficient immunity, and may have the potential risk of pathogenicity with live attenuated vaccines reverting to virulence [1,2]. In addition, parenteral immunization often involves laborious and time-consuming procedures, produces inflammatory reactions at the injection site, and creates stress in animals [3]. Although an improvement in animal and public health has been attributed to the use of conventional vaccines, they are far from ideal. Therefore, novel vaccines and alternative routes of administration are required to solve future challenges in the control of animal and human health.

Live delivery systems seem to have features of an ideal vaccine, such as the fact that they are non-pathogenic to animals and

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humans, they are easily administered, they are easy and cost effective to produce, they have an adjuvant-free status, they are stable, and they induce both mucosal and systemic immune responses when delivered orally or intranasally [2,4]. Particularly, advances in yeast biotechnology have been studied as a potential live delivery system with various advantages. Here we focus on the production of yeast-based vaccines and their applications in veterinary medicine.

2. Oral immunization

Oral administration of vaccines is an attractive alternative to parenteral vaccination, with several advantages such as improved safety for the vaccinator, vaccine, and community; better compliance with immunization schedules; the elimination of injection-site pain and infection; easier and speedier vaccine delivery; and targeted inductive sites and reduced cost [5–7]. In addition, oral administration elicits both local and systemic immune responses, leading to the effective elimination of infectious pathogens [7,8]. The adaptive humoral immune responses at the mucosal surfaces are involved in producing secretory IgA (sIgA) antibodies. sIgA binds to the microorganisms and toxins on the mucosal surface and neutralizes them by blocking their entry into the host [9,10]. Mucosal immune responses, including the oral immune response, can be stimulated to move from the inductive site to the effector site, which is generally the lamina propria on the mucosal surface, via communication among the various compartments of the common mucosal immune system (CMIS) [4,11]. Particularly, a specific mucosal immune response route can induce a more effective response at the desired effector site [4]. Oral immunization is known to induce effective responses in the gastrointestinal tract, mammary glands, and salivary glands, but is comparatively less efficient in the respiratory and reproductive tracts [4,12]. Although intranasal immunization induces sIgA antibodies in the respiratory tract, it can cause safety problems, such as redirecting vaccine antigens into the central nervous system [13]. As mentioned above, oral vaccines could be excellent candidates for pathogen protection in veterinary medicine, particularly in large herds of farmed animals.

Despite the diverse advantages of oral vaccines, they also have several problems. There are difficulties involved in collecting and processing external secretions, there are few standardized assays available, there can be an induction of tolerance, and the activity of antigens in the gastrointestinal tract is problematic [14,15]. In addition, much larger quantities of vaccine are required for oral administration than for parenteral administration, because of the low survival rates of oral vaccines in the gastrointestinal tract [16]. This could generate technical problems in terms of being able to obtain both low-cost and large volumes of oral vaccines [16].

3. Yeast as a live delivery system

Live delivery systems have been developed as one of the most effective ways to deliver vaccines to the mucosal surface [4,7,17–20]. There are four major live delivery systems that are generally used to produce vaccine antigens; bacterial, yeast, insect, mammalian, and plant expression systems [21]. Out of these delivery systems, yeast biotechnology has been investigated as a useful expression system for heterologous proteins with the particular features of yeast. The yeast-based expression system has both the advantages of prokaryotes, such as high expression levels, ease of scale-up, and low cost, and the advantages of eukaryotes such as in executing post-translational modifications (e.g. glycosylation) [22,23]. In addition, this system does not have the endotoxin problem that is associated with bacterial expression systems and viral contamination often encountered in mammalian expression systems [24]. Yeast is generally regarded as a safe organism (GRAS) for oral use, it can be used in pharmaceutical, livestock feed, and food industry applications [23], while other live bacterial carriers such as *Salmonella* spp., *Escherichia coli* spp., and *Shigella* spp. cannot be classified as safe [6]. Some yeasts such as *Pichia pastoris* have been considered the promising heterologous expression system of a protein, but the products are not regarded as GRAS, which may limit its direct application [25]. In addition, yeast cell-wall components, such as β-1,3-d-glucan, and mannan, are known to have an adjuvant potential [26]. For that reason, a number of yeasts have been studied for decades in terms of their potential for heterologous gene expression.

3.1. Yeast species for heterologous gene expression

Saccharomyces cerevisiae, *Saccharomyces boulardii*, methylotrophic yeasts such as *Hansenula polymorpha*, *P. pastoris*, and *Candida boldmu*, the budding yeast *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, and *Yarrowia lipolytica* are known to have heterologous gene-expression systems [22,27–30]. Among these yeast species, *S. cerevisiae*, *S. boulardii*, *P. pastoris*, and *K. lactis* are frequently used for the expression of heterologous genes, and for the production of therapeutic proteins.

S. cerevisiae is the most well-known and commonly used yeast in brewing and baking. It has been used for several biotechnological purposes due to its cheap and easy cultivation, well-established fermentation processes, and large-scale production capabilities [22,27]. Information on its genetics, molecular biology, and physiology has been accumulated, ensuring that this organism is a highly available eukaryotic system [31–33]. A number of selective promoter elements and mutations in *S. cerevisiae* were investigated for an increased yield or an improved quality of recombinant products [27]. Many heterologous proteins, including the first commercialized recombinant vaccine, the hepatitis B vaccine, were produced in *S. cerevisiae*, in which genetic engineering techniques were applied [27,34]. Although many heterologous proteins have been successfully expressed in *S. cerevisiae*, its limitations have meant that alternative, non-*Saccharomyces* yeasts have to be examined [27,30,35]. Generally, heterologous proteins expressed in *S. cerevisiae* have low yields, and they seem to be hyperglycosylated, which may result in differences in immunogenicity, diminished activity, or decreased serum retention of the foreign protein [35].

P. pastoris is one of the methylotrophic yeasts that uses methanol as its sole energy and carbon source [27]. The genes encoding key enzymes of the methanol-utilization pathway, which are generally used by the methylotrophic yeasts, provide inducible promoters for the efficient expression of heterologous DNA sequences [27]. In addition, *P. pastoris* produces correctly folded and secreted proteins into the medium, and is capable of performing post-translational modifications that are more similar to human protein modifications than those produced by *S. cerevisiae* [24]. Since *P. pastoris* grows on a simple mineral media and secretes only low levels of endogenous proteins, the heterologous protein comprises the major portion of the total protein in the medium, thus leading to an easier purification process [36].

K. lactis, milk yeast, is one of the few yeast species that is able to grow on lactose and whey, it is cheap and uses various substrates, as a sole source of carbon energy [37,38]. Its potential as a host for the production of heterologous proteins has been studied especially for low-value products [37]. For example, *K. lactis* can efficiently synthesize and secrete fully active foreign proteins, including prochymosin, which is poorly secreted by *S. cerevisiae* [37,39]. In addition, *K. lactis* allows rich biomass yields during fermentation at high growth rates, showing incomplete glucose repression of respiratory genes and ethanol formation, called the “Crabtree effect,” under aerobic conditions [38].