Vaccine 33 (2015) 25–33

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

A perspective on the use of *Pleurotus* for the development of convenient fungi-made oral subunit vaccines

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ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form 16 October 2014
Accepted 21 October 2014
Available online 4 November 2014

Keywords:
Adjuvant
Fungi
Glucan
Mushrooms
Oral subunit vaccine
Production cost

ABSTRACT

This review provides an outlook of the medical applications of immunomodulatory compounds taken from *Pleurotus* and proposes this fungus as a convenient host for the development of innovative vaccines. Although some fungal species, such as *Saccharomyces* and *Pichia*, occupy a relevant position in the biopharmaceutical field, these systems are essentially limited to the production of conventional expensive vaccines. Formulations made with minimally processed biomass constitute the ideal approach for developing low cost vaccines, which are urgently needed by low-income populations. The use of edible fungi has not been explored for the production and delivery of low cost vaccines, despite these organisms' attractive features. These include the fact that edible biomass can be produced at low costs in a short period of time, its high biosynthetic capacity, its production of immunomodulatory compounds, and the availability of genetic transformation methods. Perspectives associated to this biotechnological application are identified and discussed.

1. Introduction

Although vaccination represents one of the most prominent achievements in medicine, the development and exploitation of vaccines is currently hampered by the lack of knowledge of the immunoprotective antigens for some pathogens; aspects related to safety in terms of interaction of attenuated microorganisms with others that rise the possibility of virulence regression by complementation in the human body, as well as their administration risk for immunocompromised individuals, low coverage potential due to genetic variability, and high production costs [1,2]. These factors demand the development of alternative platforms for the production of subunit vaccines that are safer than those constituted by whole-killed or attenuated microorganisms, and have low production costs and feature adequate delivery strategies [3,4].

The Global Vaccine Action Plan (GVAP) of the World Health Organization is a framework to prevent millions of deaths by 2020 through providing more equitable access to existing vaccines for people in all communities. The GVAP has defined goals for the so-called decade of vaccines (2011–2020), which aim to develop and introduce new or improved vaccines and technologies as a priority objective [5].

A small number of hosts are currently used for subunit vaccine production, including insect cells, yeast, *Escherichia coli* and mammalian cell lines. Among the new platforms used for vaccine production, those consisting of low processed biomass for the formulation of economical oral vaccines have received considerable attention in the field. These platforms include yeast [6–8], plant cells [9,10], the microalgae *Chlamydomonas reinhardtii* [11], and the bacterium *Bacillus subtilis* [12]. These platforms have been implemented based on the fact that they are classified as Generally Recognized As Safe (GRAS) and produce edible biomass, which enable the production of oral vaccines under minimal biomass processing conditions, comprising freeze-drying and encapsulation in gelatin pills, resulting in an important reduction of formulation costs. The moss *Physcomitrella patens*, a non-vascular plant, has also been recently proposed for this purpose [13].

Oral vaccination constitutes a highly convenient approach for a number of reasons: (i) massive immunization programs can be better accomplished as no specialized personnel is required for administration; (ii) it is more accepted by patients due to their pain-free administration; and (iii) mucosal immune responses are evoked, which are of key relevance for the prevention of a vast number of infectious diseases. However, there are challenges for the

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http://dx.doi.org/10.1016/j.vaccine.2014.10.059
0264-410X/

successful development of oral vaccines, since the oral elicitation of robust immune responses requires a delivery system that ensures antigen stability, proper bioavailability and acceptable immunogenicity [14].

The use of edible recombinant organisms that express the antigen of interest has the advantage that a bioencapsulation effect occurs because of the complex mixture of macromolecules, such as starch, structural polysaccharides, lipids, and proteins, which protect antigen of interest from gastrointestinal enzymes and at the same time could allow for a proper antigen release. In addition, the biomass from these organisms contains compounds with biological properties, which accounts for the immunogenicity of the vaccine, and include adjuvants and mucoadhesives, such as chitosan and alginate, which are described as promising mucosal vaccine delivery systems, based on significant results generated [10,15]. Considering that the mushroom *Pleurotus ostreatus* produces edible biomass and a number of immunomodulatory compounds, this review focuses on this fungal species as a new platform for the production and delivery of recombinant subunit vaccines during the development of oral vaccines.

2. Characteristics of *P. ostreatus*

Pleurotus species are characterized by the production of fruiting bodies constituted by an eccentric stalk and a wide oyster-shell-shaped cap, with the widest portion of the cap growing away from the stalk. They grow under a wide range of temperatures and are capable of colonizing a wide spectrum of unfermented lignocellulosic wastes. Several species of the subgenus *Coremiopleurotus* are described as tree pathogens that cause the white rot of hardwood trees [16–18]. A number of species are commercially cultivated and have considerable economic value, including *P. ostreatus* (*Pleurotus florida*), *Pleurotus pulmonarius* (*Pleurotus sajor-caju*), *Pleurotus cornucopiae*, and *Pleurotus cystidiosus* [19].

Pleurotus species appear during the period from March to early November in tropical rain forests, requiring a temperature of 20–30 °C for both their vegetative growth and reproductive phase [20]. Sexual reproduction occurs through the fusion of two compatible monokaryotic hyphae (primary mycelium), followed by the differentiation of the newly formed dikaryotic hyphae (secondary mycelium), which leads to the formation of a spore-bearing sporocarp, one of the so-called fruiting bodies. The fruiting bodies carry basidia that form four haploid spores through meiosis, which germinate once more into monokaryotic hyphae to complete the life cycle (Fig. 1).

The life cycle of *Pleurotus* was described by Hilber [21]. The subgenus *Coremiopleurotus*, in which *P. cystidiosus* is a type species, has coremia, which are distinct organs engaged in the production of arthroconidia, in addition to the characteristic diploid basidioma (fruiting body), in which four haploid spores per basidium are produced [21,22]. The anamorphic organ of *P. cystidiosus* exclusively produces diploid asexual spores that may result in diploid fruit bodies [23]. The other *Pleurotus* subgenera are tetrapolar and heterothallic, and replicate through a teleomorphic (sexual) stage, producing spores through a basidiocarp.

More than 2000 edible fungal species have been described thus far. However, only about 20 of them are commercially produced [24]. Since 1980, world-wide edible mushroom cultivation has grown exponentially. In 2012, the Agricultural Marketing Resource Center [25] reported an annual U.S. production of 24,437 tons, which is 9% higher than that rendered in the previous year, generating profits of US$ 1.02 billion [26]. *P. ostreatus* is the second most cultured species. Factors behind the acceptance of the commercial consumption of the fungus known as the oyster mushroom, include the outstanding nutritional value given by its high protein content (30–37.6% w/w), its provision of all the essential amino acids, its low fat content (1.6–2.5%), fiber (9.3–13.3%), its total carbohydrate content (29.7–32.5%), and its mineral content, such as Fe, P and Zn (4.8–9.6, 12.4–15.6 and 54.6–66 mg per 100 g, respectively). It also contributes to the requirements for dairy

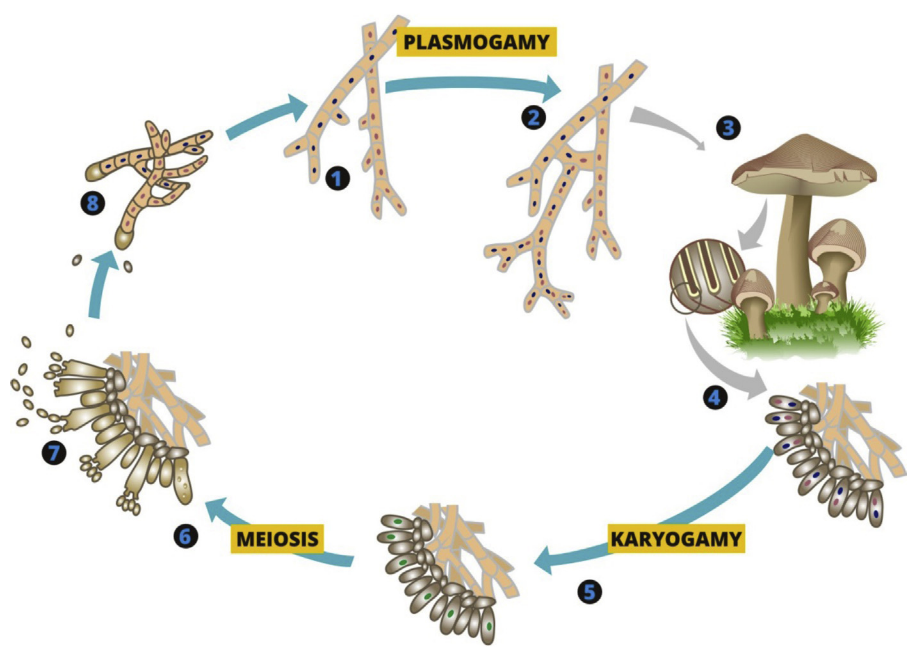

Fig. 1. Schematic representation of the *P. ostreatus* life cycle. Primary monokaryotic mycelium of different mating types (+, −) grows inside lignocellulosic-rich death matter (1); once two compatible hyphae get into close contact to each other, anastomosis takes place, giving rise to the secondary dikaryotic mycelium as a result of plasmogamy (2). When vegetative secondary mycelium reaches the surface, light and increasing oxygen availability triggers a differentiation program to develop the macroscopic reproductive structure (3), which contains basidia (4), where sporogenesis takes place by karyogamy (5) and subsequent meiosis (6) Each basidium originates four monokaryotic haploid spores of both mating types, stored outside basidium, facilitating spreading (7). When finding adequate environmental conditions, + and − spores germinate, producing primary mycelium which completes the life cycle (8).