Biotechnology Advances 32 (2014) 1382–1395

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Escherichia coli as a production host for novel enzymes from basidiomycota

Katerina Zelena *, Nadine Eisele, Ralf G. Berger

Gottfried Wilhelm Leibniz University Hannover, Institute of Food Chemistry, Callinstr. 5, D-30167 Hannover, Germany

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form 14 August 2014
Accepted 25 August 2014
Available online 2 September 2014

Keywords:
Basidiomycota
Escherichia coli
Heterologous expression
Hydrolase
Oxidoreductase
Refolding

ABSTRACT

Many enzymes from basidiomycota have been identified and more recently characterized on the molecular level. This report summarizes the potential biotechnological applications of these enzymes and evaluates recent advances in their heterologous expression in *Escherichia coli*. Being one of the most widely used hosts for the production of recombinant proteins, there are, however, recurrent problems of recovering substantial yields of correctly folded and active enzymes. Various strategies for the efficient production of recombinant proteins from basidiomycetous fungi are reviewed including the current knowledge on vectors and expression strains, as well as methods for enhancing the solubility of target expression products and their purification. Research efforts towards the refolding of recombinant oxidoreductases and hydrolases are presented to illustrate successful production strategies.

Contents

Introduction

Together with the ascomycota, basidiomycota constitute the subkingdom dikarya within the kingdom fungi. Many of these "higher fungi" are xylotrophic and grow either saprotrophically on forest detritus, leaf litter, and wood, or symbiotically (mycorrhizae). The vegetative mycelia spread out in the subterranean sphere. Governed by environmental factors, they may switch to a sexual stage and develop typical fruiting bodies carrying the basidiospores. These are reproductive cells, attached to pillar-like sterigmata protruding from the basidium, which are dispersed by wind, rain or insects. Basidiomycota not only

* Corresponding author. Tel.: +49 511 762 17257; fax: +49 511 762 4547.
E-mail address: kateryna.zelena@lci.uni-hannover.de (K. Zelena).

http://dx.doi.org/10.1016/j.biotechadv.2014.08.006
0734-9750/

produce unique small and medium molecular mass metabolites, such as volatile flavors (Berger et al., 2010), uncommon phenylhydrazines, azo-pigments (Kerschensteiner et al., 2011), antibiotics (Biswas and Bag, 2010) and glucans (Bobovčák et al., 2010), but also secrete a unique set of lignin modifying enzymes. These enable them to thrive on wood (Erjavec et al., 2012; Hoffmeister and Keller, 2007; Zorn et al., 2005b). This composite polymer is the most abundant and the most recalcitrant organic material on earth. White rot fungi preferably attack the lignin portion, leaving behind crystalline cellulose, while brown rot fungi rather attack the polysaccharides.

The economical importance is currently restricted to the agro-industry (*button mushroom, Shiitake*) and to Asian folk medicine (for example, *Ganoderma lucidum*). Formerly, the *in vitro* cultivation was thought to be complicated and not well reproducible. Today, submerged cultivation in shake flasks and even in large stirred-tank reactors for the rapid production of large amounts of homogeneous biomass has become routine. The metabolism of these highly adaptable species is controlled by environmental factors, such as nutrient and precursor availability.

The supernatant of liquid cultures typically contains numerous lignocellulose degrading enzymes, such as lignin peroxidases, manganese peroxidases, versatile peroxidases, and phenol oxidases, such as laccases, and also amidases, esterases and glycosidases (Bouws et al., 2008). Unique lipophilic oxidoreductases, such as dioxygenases or peroxygenases attack chemically inert hydrogens and thus convert hydrocarbons to functionalized derivatives (M. Fraatz et al., 2009), or remediate pollutants, such as toluene or other stable aromatics (Kinne et al., 2010). Some of the basidiomycetous esterases handle bulky substrates, such as cutin (Merz et al., 2011) or xanthophyll esters (Zorn et al., 2005b); some of the peptidases accept insoluble wheat gluten (Grimrath et al., 2011) or κ-casein as substrates (Abd El-Baky et al., 2011a). Industrially important traits, such as salt or thermotolerance, were reported (Guo et al., 2011). Stability towards enhanced pressure is uncommon for most enzymes (Eisenmenger and Reyes-De-Corcuera, 2009), but a peroxidase MsP1 of the edible garlic mushroom *Marasmius scorodonius* was highly pressure tolerant: its activity increased by a factor of 2 until about 500 bar, and at about 2 kbar the activity was still as high as under ambient pressure (Puhse et al., 2009). Considering the non-phototrophic nature of basidiomycota, the UV-B induced enzymatic formation of raspberry ketone was likewise unexpected (Taupp et al., 2008).

Digging into the complex secretome of basidiomycota is supported by the increasing availability of annotated genomes (basidionet.de/genomprojekte.html?&L=1) and sophisticated bioinformation software. A few laccases for paper processing and for the degradation of dyes and a versatile peroxidase ("Maxibright," WO 2007006792) have recently become commercial. However, to introduce new basidiomycetous enzymes into the market, production systems for the efficient and functional expression of the eukaryotic genes, such as heterologous expression systems, will be required. Once established, the recombinant platforms will not only alleviate the problem of comparably low space/time-yields of many of the wild strains, but will be indispensible tools for homology, structure-activity and binding studies and for substituting the wild-type enzymes by variants improved by directed evolution (Festa et al., 2008).

This review gives an overview on the state-of-the-art expression of recombinant enzymes from basidiomycota in *Escherichia coli*. As an expression host, *E. coli* was used mainly for the cost-efficient production of high amounts of proteins with a simple structure and a smaller molecular mass. Nowadays, plenty of tools exist facilitating the production of more complex proteins in this organism, too. An attempt in *E. coli* is usually the first step to heterologously express a target gene. Alternative systems are employed, if the product is biologically inactive, incorrectly folded or the recovery is too low (Samuelson, 2011). As a system of "first choice," *E. coli* has been used for a long time due to its rapid growth, capacity for continuous fermentation, and relatively low cost. Many expression systems designed for various applications and compatibilities are commercially available (Francis and Page, 2010; Samuelson, 2011). From the different proteins characterized for basidiomycota, two large enzymes classes—oxidoreductases and hydrolases—are mostly investigated and heterologously expressed in *E. coli*.

Heterologous production of enzymes from different classes

Oxidoreductases

Lignolytic enzymes

Peroxidases, laccases and aryl alcohol oxidases (Fig. 1) belong to the lignolytic enzymes of basidiomycota. Their expression is a highly complex process. Specific response elements, such as MRE (metal responsive element), HSE (heat shock responsive element), XRE (xenobiotic responsive element), ARE (antioxidant responsive element) in the promoter sequence of lignin degrading enzymes as well as the concentration, ratio and availability of nitrogen and carbon sources have crucial impact by the success on the enzyme expression by fungi (Janusz et al., 2013).

Peroxidases utilize hydrogen peroxide as a co-substrate for the catalytic oxidation of the diverse organic and non-organic substrates. Those from white-rot fungi belong to the class II peroxidases and are involved in lignin degradation (Welinder, 1992). The search for new sources of biofuels has stimulated attempts to commercially utilize the unique capability of the white-rot fungi to decompose lignin-rich matter. White-rot fungi are also capable of degrading a large spectrum of pollutants, such as DDT and other pesticides, dyes, cyanides and azides (Higson, 1991; Sutherland et al., 1997). The targeted search for peroxidative activities began with the finding that *Phanerochaete chrysosporium* degraded synthetic dyes (Glenn and Gold, 1983; Lucas et al., 2008; Pelaez et al., 1995). *P. chrysosporium* has become the most intensively studied white-rot fungus and emerged to a model wood decaying organism. Secreting a wide diversity of enzymes, *P. chrysosporium* was not only investigated for the production of renewable chemicals from wood, but also for the degradation of persistent environmental pollutants (MacDonald et al., 2012; Singh and Chen, 2008). Not surprisingly, the first experiments towards the expression of individual lignolytic enzymes were carried out about 30 years ago, aiming at peroxidases of *P. chrysosporium* (Table 1).

White-rot fungi are regarded as a eco-friendly alternative to problematic bleaching processes in the paper and fiber industry (Bajpai et al., 2006). *Pleurotus ostreatus* Eger EM 1303 (Florida) decolorized distillery outflows and oxidizing xenobiotics (Pant and Adholeya, 2007; Shoda, 2003). The capability of these fungi to degrade synthetic dyes, food colorants, molasses, organic halogens, lignin, and production drains was recurrently assigned to the expression of peroxidases.

Around 1000 species among the basidiomycota are edible. Enzymes from these species (which are food) receive particular attention by the food industry. An example is *M. scorodonius*, the garlic mushroom, which secreted two dye decolorizing peroxidases (DyP). The enzymes degraded tetraterpenoids to C13-norisoprenoid flavors, such as α- und β-ionone (Scheibner et al., 2008). Their woody, flowery and fruity odors in combination with low sensory thresholds turn them into most appreciated targets of aroma biotechnology.

Despite of the obvious potential, the current application of fungal peroxidases on a larger scale suffers from a single, but severe drawback: the limited availability. Lignolytic enzymes are often produced by the wild-type strain with small yields (Reddy and D´Souza, 1994). Peroxidases, in addition, may be inactivated by the co-substrate H_2O_2, by a destruction of the porphyrin cycle, or by ubiquitous peptidases and inhibitors (van de Velde et al., 2001). Taken together, these effects call for a constant replenishment, particularly in continuous processes. To accumulate sufficient catalytic activity and to increase the operational stability of peroxidases, the heterologous expression, followed by directed or random mutagenesis, represent useful tools. Considering to