Journal of Molecular Catalysis B: Enzymatic 101 (2014) 148–158

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Review

Lipase from *Yarrowia lipolytica*: Production, characterization and application as an industrial biocatalyst

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ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form
28 November 2013
Accepted 30 November 2013
Available online 7 December 2013

Keywords:
Yarrowia lipolytica lipase
Enzyme production
Separation and purification
Immobilization
Biotechnological applications

ABSTRACT

Yarrowia lipolytica (YL) is a "non-conventional" yeast that is capable of producing important metabolites. One of the most important products secreted by this microorganism is lipase, a ubiquitous enzyme that has considerable industrial potential and can be used as a biocatalyst in the pharmaceutical, food and environmental industries. This review discusses the efforts that have been made to use YL lipase as an industrial biocatalyst, including enzyme production and recovery. Special attention is given to a compilation of relevant information on enzyme separation and purification, since it is a key step for LYL application, that is not detailed in other reviews in the field. Because immobilized enzymes are preferentially used as commercial lipases, the methods of immobilization are also discussed and important biotechnological applications are addressed.

Contents

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http://dx.doi.org/10.1016/j.molcatb.2013.11.016

1. YL lipases as biocatalysts

Lipases have emerged as one of the leading biocatalysts with proven potential for contributing to the multibillion-dollar lipid technology bio-industry [1]. *Yarrowia lipolytica* has been considered an industrial workhorse [2] because of its ability to produce important metabolites and intense secretory activity. One of the most important products secreted by this microorganism is lipase. This review aims to discuss the production, biochemical characterization, immobilization and biotechnological application of these enzymes synthesized by *Y. lipolytica*.

1.1. Enzyme production

1.1.1. Influence of media composition and presence of hydrophobic substrates

Several works in the literature have demonstrated the influence of media composition and environmental conditions on lipase biosynthesis [3–6]. A review by Fickers et al. [7] notes that glucose, glycerol or mineral nitrogen compounds repressed lipase production. However, Amaral et al. [8] detected similar extracellular lipase activities for media containing glucose or olive oil as the carbon source using a second liquid phase that dissolves much more oxygen than water (perfluorodecalin) during the growth of *Y. lipolytica*. With this multiphase system the authors showed that the enzyme productivity could be enhanced 23 fold and also showed that the presence of perfluorodecalin benefitted lipase production in addition to its secretion in the extracellular medium [9]. Because perfluorodecalin has been proved to increase oxygen transfer to the aqueous phase [10], these studies show that it is an important governing factor for lipase yield. In fact, it has been demonstrated that dissolved oxygen is the main chemical engineering parameter that influences the physiological mechanisms involved during lipase synthesis [11]. Indeed, oxygen limitation tends to decrease the activity of the Lip2 promoter, which is responsible for the production of the main extracellular lipase of *Y. lipolytica* [12].

Lipase production is significantly increased in the presence of hydrophobic substrates such as oils, fatty acids and methyl esters [7,13]. Among several tested plant oils, olive oil proved to be the best raw material for lipase production by *Y. lipolytica* DSM 3286 [5]. Kebabci and Cihangir [6] have also verified that olive oil favored high lipase activity for three different *Y. lipolytica* strains. The used of inducers (triglycerides or fatty acids, as well as surfactants) is described in order to enhance microbial lipolytic activity and some papers report the use of olive oil as the inducer [14–16]. Furthermore, olive oil is described as both a carbon and inducer source by the literature [17]. In addition to olive oil, oleic acid is also considered a good carbon source for lipase production by this yeast [4]. Lipases can even be produced with raw glycerol as the carbon source when olive oil is added as an inducer [18]. Although lipidic carbon sources seem to be important for lipase production, nitrogen sources should also be carefully considered [1]. Peptone, casein, yeast extract and tryptone have been reported suitable for high extracellular lipase production; conversely, inorganic compounds do not trigger lipase synthesis [4,5].

Y. lipolytica strains can produce not only extracellular types of lipase but also cell-bound types. Indeed, the latter are only secreted to the culture medium when the carbon source becomes scarce, i.e. in the stationary phase [17]. Therefore, surfactants (such as Tween 80) can also be used in the medium composition to increase the release of the cell-bound catalyst [3].

Solid-state fermentation has also been used to successfully produce lipase. Imandi et al. [19] have worked with palm kernel cake and obtained 18.58 units of lipase activity per gram of dry fermented substrate with a 70% moisture content. Lopes et al. [20] have studied the ability of two different wild-type strains of *Y. lipolytica* to grow on olive mill wastewater (OMW). The strain W29 showed the highest potential for extracellular lipase production from OMW.

1.1.2. Enzyme production in a bioreactor

Kar et al. [11,12] investigated lipase production by *Y. lipolytica* in a 20-L batch reactor and different scaled-down apparatuses, which had been designed to reproduce the hydrodynamic phenomena encountered in large-scale equipment. Lipase production under oscillating dissolved oxygen tension significantly influenced the lipase gene expression. Furthermore, considering the nature of the substrate (lipidic) and the capacity for protein excretion and biosurfactant production of *Y. lipolytica*, intensification the oxygen transfer rate is accompanied by an excessive formation of foam, which was controlled using a mechanical foam control method [21]. This system was designed to induce the formation of a persistent foam layer in the bioreactor that led to the segregation of microbial cells, inducing a reduction in lipase yield.

Fickers et al. [22] reported the development of a process for extracellular lipase production in a 2000-L bioreactor that yielded a lipase activity of approximately 1100 U/mL after 53 h of fermentation. This finding demonstrates that the lipase production was adequately scaled-up and that its yields were comparable to those of the bench-scale process.

Furthermore, batch, fed-batch and continuous operations in a stirred-tank bioreactor to produce lipase were investigated by Deive et al. [14] and the authors demonstrated the success of the continuous process by regular enzyme production, good operational stability and mathematical models that closely approximated the experimental results.

1.2. Structural features of LYL

A recent genome survey of *Y. lipolytica* revealed 25 putative lipases [23]. Lip2 is the major extracellular lipase to have been isolated and characterized [24]. In addition to Lip2, five more lipases, Lip7, Lip8, Lip9, Lip 11, Lip12, Lip14 and Lip 18, have also been purified from *Y. lipolytica* and characterized [23,25–27].

In 2000, Pignede et al. [24] isolated the Lip2 gene from *Y. lipolytica* and showed that it encodes a 334-amino-acid precursor protein. The secreted lipase is a 301-amino-acid glycosylated polypeptide that is a member of the triacylglycerol hydrolase family (EC 3.1.1.3). Studies of the 3D structures of many lipases have shown that a 'lid' controls substrate access to the active site [28]. Generally, lipase-catalyzed reactions only occur at lipid–water interfaces. Therefore, interfacial activation has long been employed to improve the catalytic performance of a lipase. Recently, the Lip2 3D structure was solved [29], see Fig. 1, and it revealed a high homology with lipases