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Review

Glycerol as a promising substrate for *Yarrowia lipolytica* biotechnological applications

Anita Rywińska, Piotr Juszczyk, Maria Wojtatowicz, Malgorzata Robak, Zbigniew Lazar, Ludwika Tomaszewska, Waldemar Rymowicz*

Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences, Chełmońskiego 37/41, 51-630 Wrocław, Poland

ARTICLE INFO

Article history:
Received 21 June 2012
Received in revised form
14 September 2012
Accepted 26 November 2012
Available online 23 December 2012

Keywords:
Yarrowia lipolytica
Glycerol
Citric acid
Pyruvic acid
α-ketoglutaric acid
Erythritol

ABSTRACT

Unconventional and nonpathogenic *Yarrowia lipolytica* yeast has been addressed in various studies conducted in many research centers, and in recent years has been perceived as an especially attractive host for many applications of glycerol. In its initial paragraphs, this review article provides a short characteristics of *Y. lipolytica*; followed by biodiesel production and brief characteristics of crude glycerol. Further on, this review summarizes relevant scientific research concerning the conversion of crude glycerol discharged after bio-diesel (fatty acid methyl/ethyl esters) manufacturing process into value-added products through biological methods with *Y. lipolytica* yeast. The feasibility of using *Y. lipolytica* biomass, rich in proteins and oils, as food and feed additives is described as well. Subsequently, different strategies employed to produce and improve yield and productivity of organic acids (citric, pyruvic and α-ketoglutaric acid) are presented. And, finally, the biosynthesis of new products, such as erythritol, mannitol and invertase, whose synthesis from glycerol by *Y. lipolytica* would be advantageous when compared with their production from common sugars, is evaluated. In conclusion, an actual wide range of compounds that can be produced from glycerol by *Y. lipolytica* are shown to be a valuable contribution to the development of the biodiesel industry as well as a cost-effective fermentation based on renewable resources.

1. Introduction

Yarrowia lipolytica yeast is physiologically very distant from *Saccharomyces cerevisiae*. It is taxonomically assigned to the class Hemiascomycetes and the *Dipodascacea* family [1]. Previously, it occurred under various names, i.e. *Candida*, *Endomycopsis* and *Saccharomycopsis lipolytica* [2,3]. It has long been the only known taxon in the genus *Yarrowia* but recently a few *Candida* species, including *C. deformans*, *C. galli*, *C. yakushimensis* nom. inval and three novel ones: *Candida oslonensis* sp. nov., *Candida alimentaria* sp. nov., and *Candida hollandica* sp. nov., have been proposed as new members of

* *Corresponding author.*
E-mail addresses: Anita.Rywinska@wnoz.up.wroc.pl (A. Rywińska), Piotr.Juszczyk@wnoz.up.wroc.pl (P. Juszczyk), maria.wojtatowicz@wnoz.up.wroc.pl (M. Wojtatowicz), malgorzata.robak@up.wroc.pl (M. Robak), zbigniew.lazar@up.wroc.pl (Z. Lazar), ludwika.tomaszewska@gmail.com (L. Tomaszewska), Waldemar.Rymowicz@wnoz.up.wroc.pl (W. Rymowicz).
0961-9534/$ – see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.biombioe.2012.11.021

the *Yarrowia* clade [4,5]. In addition, karyotyping and RAPD-PCR analyses reveal a significant genome variability of *Y. lipolytica* strains originating from different geographic regions [6].

The genome of *Y. lipolytica* was sequenced ten years ago [7,8], and the most important unusual characteristics of this genome are: the size (20.5 Mbp, twice the size of *S. cerevisiae* genome), relatively high contents of G + C (49%), atypical structure of chromosomal centromeres and replication origins, a high number of tRNA genes (510), 5SrRNA genes dispersed throughout the whole genome (109 copies), 6449 protein-coding genes (one ORF per 3 kbp), and 1083 introns [9]. A new organization of RNA genes was reported by Acker et al. [10]. It is noteworthy that among the known ORFs there predominate genes coding enzymes of n-alkanes, fats and fatty acid metabolism [11]. Nowadays, the importance is also given to the verification of ORFs by cDNA library sequencing [12] or genes deletion [13], and to the study of new aspects of gene expression regulation [9,14,15].

Y. lipolytica is an obligate aerobe that can use only a few compounds commonly tested in yeast classifications as carbon sources [1,16]. At the same time, it possesses a unique among yeasts capability to metabolize hydrophobic substrates, including n-alkanes, oils, fats and fatty acids [3,17]. Strains of *Y. lipolytica* are isolated from dairy products (cheese, yogurt, kefir), meat, poultry and shrimp salads or from hydrocarbon- and oil-polluted environments [3,17,18]. Most isolates are haploids, yet stable diploids also exist and can be induced to form four-spored asci. In nature, the yeast grow as a mixture of budding cells, pseudohyphae and true septated hyphae. Dimorphic transition of a yeast form to mycelium (or *vice versa*) can be easily induced *in vitro* by modifying pH and temperature, carbon and nitrogen sources or the presence of specific compounds in culture media [19–22]. Two signal transduction mechanisms are involved in *Y. lipolytica* morphogenesis: the mitogen-activated kinase (MAPK) and the cyclic-AMP depended protein kinase (PKA) pathways; the first one is necessary for hyphal growth, whereas the second one is related to yeast-like growth [23,24].

The first interest in this yeast has been observed in late-sixties because of its ability to utilize n-paraffin, owing to which it has for long been used to produce single-cell proteins from crude oils containing long-chain hydrocarbons [25]. Then, it was noticed that strains of *Y. lipolytica* can produce relatively large amounts of organic acids such as citric acid and α-ketoglutaric acid when grown on these substrates [26–28]. These findings became an impulse to start the study on *Y. lipolytica* at our department [29–32].

Y. lipolytica is also known as an extracellular protein-secreting species [33–35]. The strains belonging to the species secrete mainly proteases, lipases, RNases and other proteins. The genes coding secreted proteins served to develop tools of genetic engineering [for reviews see [36,37]]. More than 40 proteins of different origins were successfully expressed in that species. The Eastern Company (Taiwan) commercialized an expression kit containing vectors and recipient strains with described procedure of transformation [see http://www.yeaster.com]. In recent years, successful expressions of the following foreign genes have been reported: tyrosinase [38], epoxide hydrolase [11], thermostable esterase [39] and invertase [40]. Studies have been undertaken to improve the bioprocess of economically-interesting heterologous proteins production. Cambon et al. [41] evaluated the activity of LIP2 (mutated in position 232) of over 100 transformants showing the utility of zeta docking platform for additional genes integration. The zeta containing *Y. lipolytica* strain was constructed by Bordes et al. [42] and the auto-cloning based strategy was described by Pignede et al. [43]. Instead, Gasmi et al. [44,45] described molecular and medium composition aspects of optimizing human interferon (hINF α2b) production by genetic engineering strains of that species.

In thus far biotechnological applications of *Y. lipolytica* involving the production of yeast biomass rich in proteins or oils, organic acids, enzymes and heterologous proteins, use has been made of a variety of carbon sources, including sugars, alkanes, plant oils, starch hydrolyzates and alcohols. More information in this respect has been provided in reviews by: Fickers et al. [17,46], Coelho et al. [47], Bankar et al. [48], Beopoulos et al. [49], Thevenieau et al. [11], and Finogenova et al. [50]. Recently, waste glycerol derived from biodiesel production has become a promising renewable substrate for microbial production. In view of the above, this manuscript reviews results of so far published investigations addressing glycerol utilization by *Y. lipolytica* in various bioprocesses.

2. Glycerol – a promising substrate for biotechnological applications

Biodiesel (fatty acid methyl esters – FAME) is an alternative diesel fuel made of vegetable oil, animal fat, cooking oil, waste grease, or other suitable lipid feedstock. Typical crude materials of biodiesel are rapeseed oil, canola oil, soybean oil, sunflower oil, palm oil and oil from microalgae [51]. Beef and sheep tallow and poultry oil from animal sources and waste cooking oil, fish oil, jatropha oil, and coconut are also sources of crude materials [52,53]. In Europe, rapeseed oil is the major component in biodiesel production, whilst in Brazil, oils from soybean and sunflower. African palm oil, castor and *Jatropha curcas* are used as well [54]. According to the European Biodiesel Board, in 2011 the total European biodiesel production capacity reached 22 million tones [see http://www.ebb-eu.org]. Biodiesel is produced through transesterification of lipid with a simple alcohol, such as methanol or ethanol (alcoholysis), generally catalyzed by NaOH and KOH or acid [55,56]. Chemical transesterification using an alkali-catalysis process yields high conversion levels of triglycerides to their corresponding methyl or ethyl esters in short reaction times, and crude glycerol is the major by-product of this reaction. It was estimated that approximately 1 kg of crude glycerol is generated for every 10 kg of biodiesel produced [57]. Thus, today roughly two million tones of additional crude glycerol are available in Europe. The recovery of crude glycerol is difficult because it is obtained from various feedstocks used to produce biodiesel and additionally it contains different levels of various contaminants, mainly alkali soaps, methanol, hydroxides, methyl esters, microelements (iron, magnesium, calcium, zinc), nitrogen, phosphorus, and water [58]. Depending on the catalyst used for the biodiesel reaction, the crude glycerol may contain 6–8% of inorganic salts. Several