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Review

Yarrowia lipolytica as a biotechnological chassis to produce usual and unusual fatty acids

Rodrigo Ledesma-Amaro *, Jean-Marc Nicaud *

[a] INRA, UMR1319 Micalis, F-78350 Jouy-en-Josas, France
[b] AgroParisTech, UMR Micalis, Jouy-en-Josas, France

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ABSTRACT

One of the most promising alternatives to petroleum for the production of fuels and chemicals is bio-oil based chemistry. Microbial oils are gaining importance because they can be engineered to accumulate lipids enriched in desired fatty acids. These specific lipids are closer to the commercialized product, therefore reducing pollutants and costly chemical steps. *Yarrowia lipolytica* is the most widely studied and engineered oleaginous yeast. Different molecular and bioinformatics tools permit systems metabolic engineering strategies in this yeast, which can produce usual and unusual fatty acids. Usual fatty acids, those usually found in triacylglycerol, accumulate through the action of several pathways, such as fatty acid/triacylglycerol synthesis, transport and degradation. Unusual fatty acids are enzymatic modifications of usual fatty acids to produce compounds that are not naturally synthetized in the host. Recently, the metabolic engineering of microorganisms has produced different unusual fatty acids, such as building block ricinoleic acid and nutraceuticals such as conjugated linoleic acid or polyunsaturated fatty acids. Additionally, microbial sources are preferred hosts for the production of fatty acid-derived compounds such as γ-decalactone, hexanal and dicarboxylic acids. The variety of lipids produced by oleaginous microorganisms is expected to rise in the coming years to cope with the increasing demand.

Contents

Abbreviations: GEMs, Genome scale metabolic models; MFA, metabolic flux analysis; TAG, triacylglycerol; DAG, diacylglycerol; CLA, conjutated linoleic acid; EPA, eicosapentanoic acid; DHA, docosahexanoic acid; LA, linoleic acid; ALA, alpha-linoleic acid; RA, ricinoleic acid; DCW, dry cell weight; DCA, dicarboxyilic acid.

* Corresponding authors at: Institut Micalis, INRA-AgroParisTech, UMR1319, Team BIMLip: Biologie Intégrative du Métabolisme Lipidique, CBAI, F-78850 Thiverval-Grignon, France.
E-mail addresses: Rodrigo.Ledesma@grignon.inra.fr (R. Ledesma-Amaro), jean-marc.nicaud@grignon.inra.fr (J.-M. Nicaud).

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1. Introduction

General concerns about climate change and the increasing search for renewable sources of energy are boosting bio-lipids as promising alternatives to fossil fuels. Over the last few decades, the chemical industry has been developing technology that uses oils and fats as renewable sources of precursors in the synthesis of polymers, plasticizers, lubricants, coatings, surfactants, drugs, and fuels, among others [1]. Normally, lipids are produced in animals, plants and microbes in the form of triacylglycerols (TAGs), which are thereafter modified by chemical processes such as oxidation, C–C bond-forming additions, metathesis or C–H activation [2] to constitute the final products.

Notably, in contrast to animals and plants, microorganisms can be easily engineered and accepted by industry and society. The recent developments in systems metabolic engineering—an emergent discipline that combines synthetic and systems biology and metabolic modeling with traditional metabolic, genetic and protein engineering techniques—facilitates the modification of microorganisms to directly produce unusual fatty acids. These unusual fatty acids are one step closer to the final product, thus reducing or completely avoiding chemical processes during the manufacture [1].

In the recent years, the bio-based production of unusual fatty acids has attracted significant attention in both plants [3] and microbial sources (see below).

Some microorganisms, called 'oleaginous microorganisms', are able to accumulate high amounts of usual lipids (more than 20% of their cell dry weight), and they are considered the most suitable chassis for bio-oil production. Nonetheless, several approaches have been employed to convert model organisms into oleaginous organisms by genetic engineering. For example, *E. coli* has been extensively used to produce free fatty acids [4], *S. cerevisiae* has been engineered to be able to accumulate up to 50% of its DCW as lipids [5], and *A. gossypii* modified to harbor genes from oleaginous organisms was able to accumulate up to 70% of lipids [6].

Among oleaginous microorganisms, *Yarrowia lipolytica* is the most studied due to both its interesting biotechnological characteristics and its suitability to be manipulated in the laboratory. Here, we review the lipid metabolism of *Y. lipolytica*, the latest advances and techniques in its manipulation and the different approaches employed in recent years to produce unusual fatty acids.

2. *Yarrowia lipolytica*: an industrial yeast

Yarrowia lipolytica is a dimorphic, non-pathogenic ascomycetous yeast. It is often found in environments with the presence of hydrophobic substrates such as dairy products and oily waste. Therefore, several strains have been isolated in soils contaminated with oils, in marine environments, in sediment samples and in waste waters [7]. Importantly, the safety of this yeast has recently been assessed [8] because food supplements have recently been approved for commercialization [9]. This safety approval is particularly important for broadening the range of possible applications of the products derived from the fermentation of *Yarrowia lipolytica*.

The safety status of *Yarrowia lipolytica* and its unique physiological features, such as the growth in hydrophobic substrates, has made it an important biotechnological yeast. *Yarrowia lipolytica* has been used for many food-related applications [10], such as the production of meat and dairy products, the production of aromas such as gamma decalactone, the synthesis of organic acids (citric, isocitric, alpha-ketoglutaric, pyruvic and succinic), spoilage yeasts for discoloration, the production of polyalcohol for sucrose replacement, the production of emulsifiers and surfactants and the production of single cell oils and proteins for food. It has also been proposed for the treatment and degradation of pollutants [7] such as hydrocarbons, oils, nitro, halogenated and organophosphate compounds, for the reduction of metals and for the treatment of wastewater.

Additionally, it is an advantageous host for the production of proteins, and more than 130 different proteins with academic or commercial applications have been produced in this yeast [11].

Finally, as we introduce above and describe below, *Yarrowia lipolytica* has been widely used in the production of lipids and lipid-derived compounds [12] such as biodiesel, edible oils or dicarboxylic acids as building blocks for polymers.

3. Systems metabolic engineering tools

The huge number of industrial applications has boosted both basic research to understand the physiological features of *Y. lipolytica* and the development of metabolic engineering tools. The genome of this yeast (strain W9, CLIB122) has been sequenced [13] and manually annotated by a network of expert curators in the program Génolevures [14]. Additionally, the genome of the strain Po1f, commonly used in metabolic engineering approaches, has also been recently sequenced [15].

For decades, molecular biology techniques have been developed in *Y. lipolytica*. It has been engineered to increase the efficiency of homologous recombination to improve the frequency of the transformation, which has facilitated gene overexpression and deletion [16]. Examples of molecular biology tools developed in this yeast are replicative plasmids, constructions for genomic insertions, constitutive and inducible promoters, florescence tags, protein expression and secretion vectors. A recent review covering the topic was published by Madzak [17]. These molecular biology tools, along with systems biology, synthetic biology and mathematical models, permit systems metabolic engineering, which is currently boosting industrial biotechnology [18].

Genome scale metabolic models (GEMs) have been shown to be useful tools in both basic and applied research [19]. Accordingly, they help in the re-annotation of genomes and metabolic network analysis, and they also permit the identification of non-obvious targets for metabolic engineering. In recent years, three genome-scale metabolic models of *Y. lipolytica* have been developed [20–22], although none of them has yet been used in metabolic engineering approaches.

Synthetic biology has been defined as 'the design and construction of new biological systems (e.g., genetic control systems, metabolic pathways, chromosomes, cells) that do not exist in nature through the assembly of well-characterized, standardized, reusable components' [23]. Therefore, novel tools must be developed to facilitate and to increase the complexity of synthetic systems. In *Yarrowia lipolytica*, a method for the one-step integration of multiple genes has been established [24]. Additionally, synthetic terminators [25] and plasmids have been developed for increased expression levels and copy numbers [26] and hybrid synthetic promoters [27,28]. In recent years, the CRISPR-Cas9 system, a tool for genome engineering, has been developed and shown to be useful for manipulating a wide range of organisms, including yeasts [29,30]. This technology has not yet been fully implemented in *Yarrowia*, but this is expected to change in the near future [31].

High-throughput technologies have enabled the analysis of large amount of omics data for investigating cellular metabolism and physiology at the systems level [32]. These systems biology data have recently been obtained and analyzed for *Y. lipolytica*. In this regard, transcriptomic analysis revealed four different transcription profiles over 32 h of fermentation and identified genes potentially involved in the metabolism of oleaginous species [33]. Additionally, transcriptomic and proteomic analyses of *Y. lipolytica* have been conducted in relation