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Review

Yarrowia lipolytica as a model for bio-oil production

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ABSTRACT

The yeast *Yarrowia lipolytica* has developed very efficient mechanisms for breaking down and using hydrophobic substrates. It is considered an oleaginous yeast, based on its ability to accumulate large amounts of lipids. Completion of the sequencing of the *Y. lipolytica* genome and the existence of suitable tools for genetic manipulation have made it possible to use the metabolic function of this species for biotechnological applications. In this review, we describe the coordinated pathways of lipid metabolism, storage and mobilization in this yeast, focusing in particular on the roles and regulation of the various enzymes and organelles involved in these processes. The physiological responses of *Y. lipolytica* to hydrophobic substrates include surface-mediated and direct interfacial transport processes, the production of biosurfactants, hydrophobization of the cytoplasmic membrane and the formation of protrusions. We also discuss culture conditions, including the mode of culture control and the culture medium, as these conditions can be modified to enhance the accumulation of lipids with a specific composition and to identify links between various biological processes occurring in the cells of this yeast. Examples are presented demonstrating the potential use of *Y. lipolytica* in fatty-acid bioconversion, substrate valorization and single-cell oil production. Finally, this review also discusses recent progress in our understanding of the metabolic fate of hydrophobic compounds within the cell: their terminal oxidation, further degradation or accumulation in the form of intracellular lipid bodies.

Contents

Abbreviations: AA, arachidonic acid; ACAT, acyl-CoA:cholesterol acyltransferase; ACC, acyl-CoA carboxylase; ACL, ATP citrate lyase; AMP, adenosine monophosphate; ATP, adenosine triphosphate; DAG, diacylglycerol; DHAP, dihydroxyacetone phosphate; FFA, free fatty acids; GLA, γ-linolenic acid; G-3-P, glycerol-3-phosphate; HS, hydrophobic substrates; IMP, inosine 5′-monophosphate; LB, lipid body; MAG, monoacylglycerol; ME, malic enzyme; NADPH, nicotinamide adenine dinucleotide phosphate; PUFA, polyunsaturated fatty acids; SCO, single-cell oil; SE, steryl esters; TAG, triacylglycerols (triglycerides).

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1. Introduction

The yeast *Yarrowia lipolytica* is often found in environments rich in hydrophobic substrates, such as alkanes or lipids, and has developed sophisticated mechanisms for the efficient use of hydrophobic substrates (HS) as the sole carbon source [1,2]. One of the most striking features of this yeast is the presence in its genome of several multigene families involved in these metabolic pathways. The complexity and multiplicity of these genes enable *Y. lipolytica* to use and valorize a wide range of hydrophobic substrates (HS). Using these mechanisms, this yeast can accumulate lipids to levels exceeding 50% of cell dry weight [3]. *Y. lipolytica* may therefore be considered an oleaginous yeast. Lipid accumulation is probably enhanced by the many protrusions on the cell surface, facilitating HS uptake from the medium [4]. The internalized aliphatic chains are then broken down to meet needs for growth, or accumulate in an unchanged or modified form. These lipids form the storage lipid fraction, which consists mostly of triacylglycerols (triglycerides) (TAG) and steryl esters (SE). In addition to direct substrate assimilation from the medium, *de novo* TAG biosynthesis is another energy storage process providing fatty acids for membrane phospholipid formation. SE formation and mobilization provide the sterols required for membrane proliferation. Storage molecules accumulate in a specialized compartment of the cell known as the lipid body (LB). Yeast lipid bodies consist of a lipid core encased in a phospholipid monolayer, within which many proteins with diverse biochemical activities are embedded [5–7]. Several of these proteins metabolize lipids and the LB therefore probably plays a key role not only in lipid storage, but also in lipid biosynthesis, metabolism, degradation and substrate trafficking [6]. LB formation and function are tightly linked to the synthesis of TAG and SE. A recently identified lipid-binding protein in *Y. lipolytica* LB [8,9] has been implicated in lipid trafficking between the cytoplasm and LB, suggesting that free (non-esterified) fatty acids (FFA) probably accumulate in lipid bodies too [4,8,10,11].

A few models have been developed for the study of lipid metabolism. These models include *Saccharomyces cerevisiae*, which has long been used as a genetic model in studies that have greatly improved our understanding of lipid metabolism [12]. The enzymes involved in TAG biosynthesis, storage and degradation are very similar between species, and particularly between yeasts, but *S. cerevisiae* is not an oleaginous yeast and accumulates only moderate amounts of lipids (less than 15% of its biomass). Furthermore, unlike *S. cerevisiae*, which produces similar amounts of TAG and SE, *Y. lipolytica* stores mostly TAGs (>90%). This yeast is also unusual in accumulating significant quantities of FFA within the cell.

The unique features of *Y. lipolytica*, together with the availability of efficient genetic tools for this species, have stimulated interest in the use of this yeast as a model for bio-oil production, with great potential for biotechnological applications. Several technologies, including various fermentation configurations, have been already used for single-cell oil (SCO) production by strains of *Y. lipolytica* grown on various agro-industrial by-products or waste [2,11]. The potential applications of these processes include the production of reserve lipids with particular structures (e.g. oils enriched in essential polyunsaturated fatty acids) and the production of nonspecific oils for use as renewable starting materials for the synthesis of bio-fuels. This review aims to provide insight into the routes of biosynthesis and degradation leading to the formation of oils and an overview of recent advances in the physiology and genetics of *Y. lipolytica* relating to the assimilation of HS.

2. Lipid synthesis and accumulation factors in oleaginous microorganisms

2.1. Oleaginous yeasts

Few microorganisms are known to accumulate lipids to a significant level. Those species able to do so to a level corresponding to more than 20% of their biomass are described as oleaginous. Fewer than 30 of the 600 species of microorganisms investigated in one study were found to be oleaginous [13–15]. The best known oleaginous yeasts include genus of *Candida*, *Cryptococcus*, *Rhodotorula*, *Rhizopus*, *Trichosporon* and *Yarrowia*. On average, these yeasts accumulate lipids to a level corresponding to 40% of their biomass. However, in conditions of nutrient limitation, they may accumulate lipids to levels exceeding 70 % of their biomass (Table 1). Nevertheless, lipid content and profile differ between species. For instance, *Cryptococcus curvatus* and *Cryptococcus albidus* accumulate lipids to equivalent levels (58% and 65%, respectively), but their fatty acid profiles differ significantly. *C. curvatus* accumulates

Table 1
Lipid contents and fatty acid profiles of selected oleaginous yeasts [16]. Lipid contents are expressed, in terms of mass, as a fraction of dry cell mass (% [$g_{lip}\ g_X^{-1}$], weight/dry weight).

Species	% Lipid ($g_{lip}\ g_X^{-1}$)	Major fatty acid residues (relative% w/w)					
		C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Cryptococcus curvatus	58	25	T[a]	10	57	7	0[b]
Cryptococcus albidus	65	12	1	3	73	12	0
Candida sp. 107	42	44	5	8	31	9	1
Lipomyces starkeyi	63	34	6	5	51	3	0
Rhodotorula glutinis	72	37	1	3	47	8	0
Rhodotorula graminis	36	30	2	12	36	15	4
Rhizopus arrhizus	57	18	0	6	22	10	12
Trichosporon pullulans	65	15	0	2	57	24	1
Yarrowia lipolytica	36	11	6	1	28	51	1

[a] T means trace.
[b] 0 means none detected.