

Minireview

The 'LipoYeasts' project: using the oleaginous yeast *Yarrowia lipolytica* in combination with specific bacterial genes for the bioconversion of lipids, fats and oils into high-value products

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Summary

The oleochemical industry is currently still dominated by conventional chemistry, with biotechnology only starting to play a more prominent role, primarily with respect to the biosurfactants or lipases, e.g. as detergents, or for biofuel production. A major bottleneck for all further biotechnological applications is the problem of the initial mobilization of cheap and vastly available lipid and oil substrates, which are then to be transformed into high-value biotechnological, nutritional or pharmacological products. Under the EU-sponsored LipoYeasts project we are developing the oleaginous yeast *Yarrowia lipolytica* into a versatile and high-throughput microbial factory that, by use of specific enzymatic pathways from hydrocarbonoclastic bacteria, efficiently mobilizes lipids by directing its versatile lipid metabolism towards the production of industrially valuable lipid-derived

compounds like wax esters (WE), isoprenoid-derived compounds (carotenoids, polyenic carotenoid ester), polyhydroxyalkanoates (PHAs) and free hydroxylated fatty acids (HFAs). Different lipid stocks (petroleum, alkane, vegetable oil, fatty acid) and combinations thereof are being assessed as substrates in combination with different mutant and recombinant strains of *Y. lipolytica*, in order to modulate the composition and yields of the produced added-value products.

Lipids and biotechnology

Lipids constitute a large and diverse group of compounds, which all are united by their hydrophobic nature and consequently their solubility in non-polar organic solvents. Lipids and related lipophilic compounds are found, e.g. in animal and plant fats and oils, as waxes, phospholipids, eicosonoids, terpenes, and lipid-soluble steroids and vitamins. The main sources for the industrial use of lipids are animal fats and plant oils, with the latter ones being forecasted for 2008–2009 to be produced at a scale of 418 million tonnes worldwide (http://www.abareconomics.com/interactive/08ac_Sept/htm/oilseeds.htm).

The chemical industry has developed a number of processes for the chemical conversion of lipids into various valuable industrial end products. An overview on the plethora of chemical conversion processes that is being worked upon can be obtained from the 2008 Euro Fed Lipid Congress abstracts book (Oils, Fats, and Lipids in the third millennium, 7–10 September, 2008, Book of abstracts). For example, lipids can serve as raw materials for the production of functional polymers such as polyurethanes, polyamides and polyesters by subjecting unsaturated vegetable oils (e.g. soy or canola) to ozonolysis followed by a hydrogenation step (Narine, 2008). Or, lipid-derived fatty acid monomers are being modified by adding specific functional groups prior to their incorporation into specific polymers, such as polyesters and polyamides (Behr and Rothstock, 2008). Hydrocarbons

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for use as hydrocarbon-based biofuels can be synthesized from lipids by thermal and catalytic decarboxylation (Stadlbauer *et al.*, 2008). Rare functionalized unsaturated fatty acids can be produced from calendula and tung oils (Biermann *et al.*, 2007), while modified fatty acids with reduced *trans*-fat content can be obtained from vegetable oils via metal-based catalysis (Golden *et al.*, 2008). These and other innovative strategies for the production of added-value lipids typically still depend on costly and energy-consuming chemical reactions.

Biotechnology on the other hand provides a wide range of industrial applications that cannot easily be achieved by conventional chemistry. Although the production of biofuels from oils via lipase catalysis currently represent a major focus in the application of biotechnology to lipid transformation (Liu *et al.*, 2008; Watanabe and Shimada, 2008), new types of bioconversions are emerging, which complement pure enzymatic catalysis now by whole cell-based biocatalytic (WCB) conversion strategies. The former group of enzymatic bioconversions include, apart from lipase catalysed conversions, e.g. the biosynthesis of very-long-chain PUFAs employing desaturases and elongases (Hoppe *et al.*, 2008) or the rational application of acyl-CoA acyltransferases to modify the relative composition of sunflower oils, aiming at lower *trans*-fats content during hydrogenation process while enhancing the proportion of stearic fat (Martinez-Force *et al.*, 2008). WCB on the other hand are employed for the production of conjugated PUFAs using *Bifidobacterium* species (Gorissen *et al.*, 2008), for the production of biosurfactants from vegetable oils, often refinery wastes, using different bacteria and yeast species (Bernarski *et al.*, 2004; Raza *et al.*, 2007; Sarubbo *et al.*, 2007; reviewed in Van Bogaert *et al.*, 2007), but also for the targeted production of fine chemicals, such as vanillin from rice bran oil by using *Aspergillus niger* and *Pycnoporus cinnabarinus* (Zheng *et al.*, 2007).

Yeast strains seem to be particularly suited for the conversion of lipids. Some prominent yeast species, the so-called oleaginous yeasts that in fact were originally isolated from lipid substrates, have evolved sophisticated metabolic pathways that allow them to thrive on a variety of lipid substrates (reviewed in Thevenieau *et al.*, 2009; 2010). Yet, only few examples have been reported this far on the use of these yeasts in lipid bioconversion: *Yarrowia lipolytica* as well as some species of the genus *Candida* were employed for the lipid-derived production of biosurfactants and of gamma-decalactone, a high-value aroma substance (Endrizzi and Belin, 1995; Waché *et al.*, 2001). The notable under-utilization of oleaginous yeast for lipid bioconversion may correspond to the prominent research focus on conventional yeasts such as *Saccharomyces cerevisiae*, which are thus

much better studied in general terms. Yet, since recently the sequence of the oleaginous yeast *Y. lipolytica* has been published (Dujon *et al.*, 2004), the foundations were laid to make this yeast a prime model organism for studying lipid metabolism in oleaginous yeasts. In this review we describe targeted efforts in utilizing *Y. lipolytica* for the bioconversion of lipids into diverse high-value products, by combining the yeast's lipid-metabolizing potential with biosynthetic capacities derived from oil- and lipid-degrading bacteria.

Y. lipolytica as a versatile fermentation platform

Yarrowia lipolytica is considered a 'non-conventional' yeast primarily due to its ability to grow on a variety of substrates that are rich in lipids such as certain dairy and meat products (reviewed in Wolf, 2003; Beopoulos *et al.*, 2010). Variant strains have also been isolated from soil, sewage and oil-polluted environments (reviewed in Beopoulos *et al.*, 2010), where in the latter case its ability to grow on a variety of aliphatic hydrocarbons has been exploited for the bioremediation of oily and greasy waste waters (Oswal *et al.*, 2002; Lanciotti *et al.*, 2005). *Yarrowia* is considered a GRAS (generally recognized as safe) organism that has already been used for some biotechnological applications like, e.g. citric acid production and heterologous protein expression; however, none of these production processes has exploited lipids as substrates (Madzak *et al.*, 2004; Forster *et al.*, 2007). On the other hand, this yeast has been used to produce neutral lipids consisting of triacylglycerols (TAGs) and sterol esters from oleic acid as substrate (Mlickova *et al.*, 2004), which accumulate as intracellular lipid bodies making up for about 80% of the cell dry weight (reviewed in Fickers *et al.*, 2005; Beopoulos *et al.*, 2009). *Yarrowia*'s ability to accumulate lipids is now being explored, e.g. to produce in a targeted way either oils rich in saturated fatty acids (Papanikolaou *et al.*, 2003), or mono- and poly-unsaturated oils, by corresponding genetic engineering of the yeast (Beopoulos *et al.*, 2008).

Yarrowia's versatile lipid metabolism allows it, in response to the prevailing metabolic conditions, to either degrade, store or modify exogenous lipid substrates via distinct compartmentalized metabolic routes (reviewed in Fickers *et al.*, 2005). *Yarrowia lipolytica* shows remarkable adaptations to lipid substrates in its ability to actually communicate with lipids by producing extracellular protrusions that serve to gather lipid droplets at the cell's surface, as well as to store significant amounts of lipids intracellularly (Mlickova *et al.*, 2004). Recent research efforts directed at elucidating its lipid metabolism (reviewed in Fickers *et al.*, 2005) together with available genomic data (Dujon *et al.*, 2004) make it now possible to intercept its branched lipid metabolism for biotechnolo-

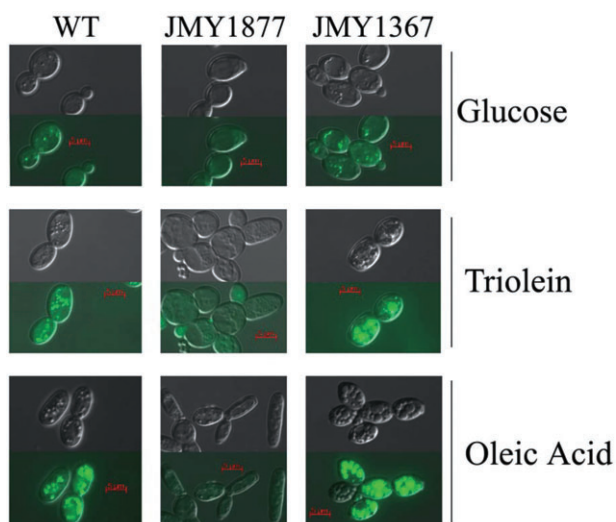


Fig. 1. Scanning electron and fluorescence microscope visualization of lipid accumulation and lipid body formation depending on genotype of *Y. lipolytica* cells grown on glucose (YPD), triglyceride (YNB-Triolein) and fatty acid (YNB-Oleic acid). Strains used are the wild type (WT), a quadruple lipid body-deficient mutant deleted for all acyl transferases ($\Delta dga1$, $\Delta lro1$, $\Delta are1$ and $\Delta are2$) (JMY1877) (A. Beopoulos, R. Haddouche, P. Kabran, T. Chardot and J.-M. Nicaud, in preparation) and an «obese» lipid-overproducing mutant strain deleted for *GUT2* and the *POX* genes (JMY1367) ($\Delta pox1-6$ and $\Delta gut2$) (Beopoulos *et al.*, 2008). Upper panel (no staining) and lower panel (lipid are stained with lipidTox™green neutral strain).

gical purposes. Figure 1 demonstrates the scope of *Y. lipolytica*'s storage capacity when grown on lipids (triolein) or lipid precursors (oleic acid) as compared with a rich non-lipid carbon source (glucose), and for different genetic backgrounds, i.e. the wild type (WT), a mutant deficient in lipid production (JMY 1877), and an 'obese' mutant (JMY 1367), where metabolic routes competing with lipid production are blocked.

Yarrowia lipolytica may gradually develop into a new yeast model organism for industrial use with gradually enhanced understanding of its genetics and physiology. A wide range of tools have by now been developed that allow genetic manipulation of *Yarrowia* to be done with the same ease as for *S. cerevisiae* (Fickers *et al.*, 2003; Madzak *et al.*, 2004). A large set of additional mutations to more specifically alter this yeast's lipid metabolism are currently being engineered by Dr Nicaud and colleagues at INRA (R. Haddouche, in preparation). These strains then serve as tool box for the rational redesign of *Yarrowia*'s lipid flux with modified β -oxidation, TAG biosynthesis and ω -oxidation thus allowing for the production of lipid precursors or for their conversion into other added-value derivatives. Additional engineering efforts can further optimize and tailor *Yarrowia*'s lipid metabolism to create new branch points for interception, and to increase metabolic flux along these engineered routes.

Hydrocarbonoclastic bacteria as sources for novel enzymes

The term 'Hydrocarbonoclastic bacteria' refers to group of marine bacteria that are capable of utilizing aliphatic hydrocarbons as their sole carbon and energy source. These bacteria are present in low abundance in marine environments but bloom after an oil spill (Golyshin *et al.*, 2003). One species, *Alcanivorax borkumensis*, appears to dominate microbial communities associated with oil-polluted waters and is assumed to be mostly responsible for the removal of marine oil contaminations (Golyshin *et al.*, 2003). This bacterium is particularly efficient at degrading a wide range of aliphatic hydrocarbons, which initiated research aimed at finding enzymes that could be used for bioremediation, biocatalysis and biosynthesis. Earlier completed genome sequence of *A. borkumensis* (Schneiker *et al.*, 2006) and its first genome and proteome analyses revealed multiple alkane degradation pathways as well as metabolic routes for the accumulation of carbon storage compounds from scavenged alkanes (Sabirova *et al.*, 2006). More recently, genome sequences of other hydrocarbonoclastic marine bacteria [*Marinobacter aquaeolei*, *Idiomarina loihiensis*, *Oleospira antarctica* (*Marinobacter* and *Oleospira* are currently being sequenced)] have become available, which provides additional sources for the identification of novel enzyme functions for the biocatalytic conversion of lipophilic compounds into valuable products, such as putative hydrolases and oxygenases.

Marine hydrocarbonoclastic bacteria have not only evolved efficient mechanisms for the degradation of aliphatic hydrocarbons, but also for the formation of storage compounds enabling them to survive periods of poor nutrient conditions (reviewed in Steinbüchel *et al.*, 1991). *Alcanivorax* has been shown to accumulate significant amounts of wax esters (WEs) and TAGs (Kalscheuer *et al.*, 2007), while *Marinobacter hydrocarbonoclasticus* is known to make WEs from recalcitrant isoprenoid alcohols that are released from accumulated (bacterio)chlorophylls in marine sediments (Rontani *et al.*, 1999). Other yet undiscovered hydrocarbonoclastic bacteria may have evolved other scavenging mechanisms that allow them to utilize other types of hydrocarbons and make new types of storage compounds, and hence it is anticipated that targeted screening and metagenomics efforts focussed on this group of marine bacteria will uncover new metabolic functions that can be exploited for the conversion of hydrocarbons and lipids into high-value products.

High-value target compounds

Research efforts under the project described here all aim at producing high-value fine chemicals, carotenoids and

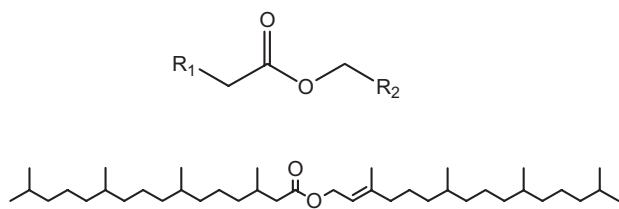


Fig. 2. Fatty acid-fatty alcohol (top) and isoprenoid WE (bottom).

biopolymers from bulk materials like cheap animal fats or vegetable oils. Some key target compounds are described in the following.

Wax esters are esters of long-chain aliphatic alcohols and fatty acids (Fig. 2) and are used as high-performance lubricants for engines, transmission and hydraulic systems (Lea, 2002). They are also used in cosmetics, foods and pharmaceuticals. Currently, these compounds are produced primarily from mineral oils, although the production of biodegradable lubricants from renewable sources increasingly attracts interest (Goodstein, 2004). However, as the most accessible natural sources of WEs, whale sperm oil and jojoba oil, are too expensive for wide range use, researchers have explored microbial sources for the synthesis of bio-WEs and thus some Gram-negative bacteria such as, e.g. *Acinetobacter*, *Alcanivorax* and *Marinobacter* species have been reported to synthesize different types of WEs as carbon storage compounds (Kalscheuer, 2010).

Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers synthesized by bacteria and used as bioplastics, e.g. for a variety of packaging and medical applications. Chemically being composed of 3-hydroxy fatty acid monomers, these polymers can be classified as short-chain-length (SCL), medium-chain-length (MCL) or long-chain length (LCL) PHAs. SCL-PHAs contain monomers with a chain length of three to five carbons, while MCL-PHAs contain 3-hydroxy acid monomers with 6–16 carbon chains, and long-chain-length PHAs contain monomers longer than with 16 carbon atoms (Fig. 3). Currently, the best-characterized PHA is the SCL-PHA polyhydroxybutyrate PHB (four-carbon-chain length)

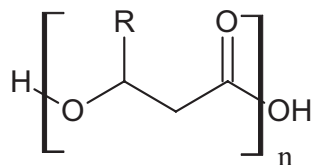


Fig. 3. Short- and medium-chain-length PHAs. SCL-PHA's: polyhydroxybutyrate (C4), polyhydroxyvalerate (C5). MCL-PHA's: poly(3-hydroxyhexanoate) (C6), poly(3-hydroxyoctanoate), poly(3-hydroxydecanoate) (C10), poly(3-hydroxydodecanoate) (C12), poly(3-hydroxytetradecanoate) (C14), and poly(3-hydroxyhexadecanoate) (C16).

which can be produced in an industrial scale. However, PHB is a rather brittle and inflexible biopolymer which compromised in its range of applications. To increase the utility of these biopolymers it is necessary to produce PHAs with different monomer compositions, e.g. longer chain length for improved mechanical properties.

Yeasts with their more active metabolism yielding PHA precursors appears to be a better choice, as compared with bacterial hosts, for large-scale PHA production, because of their ability to grow on a wider range of low-cost feedstocks and because their sizes allow easier recovery during downstream processing (Terentiev *et al.*, 2004). Recently attempts have therefore been aimed to produce PHAs in *S. cerevisiae*, *Pichia pastoris* and *Arxula adenivorans* by overexpressing either *Pseudomonas aeruginosa* PhaC synthase or the entire *phbABC* cluster of *Ralstonia eutropha* (Poirier *et al.*, 2001; 2002; Terentiev *et al.*, 2004). However, PHA production rates so far obtained in yeasts were low compared with those obtained with bacteria. *Yarrowia lipolytica* specialized in efficient mobilization, storage, and conversion of lipids into PHA, is expected to be more appropriate yeast as heterologous host for PHA production.

Hydroxylated fatty acids (HFAs) have been used as chiral building blocks for the synthesis of a number of fine chemicals (Seebach *et al.*, 1986; Lee *et al.*, 2002) (Fig. 4). Moreover, some of HFAs have high potential for antimicrobial agents or biomedical applications (Hou, 2008). Enantiomerically pure HFAs can be derived from PHAs by chemical hydrolysis, which is a not very cost-effective production route. Alternatively, HFAs can be produced from PHAs by enzymatic depolymerization using PHA depolymerases that in PHA-producing bacteria catalyse the mobilization of storage compounds for growth (Ren *et al.*, 2005). The most desirable process for HFAs production, however, would be conversion of 3-hydroxy acyl-CoA intermediates from microbial β -oxidation of fatty acids into *R*-3-hydroxy acyl acids. By employing genetic engineering of *Yarrowia*, we will target 3-hydroxy intermediates of β -oxidation into these valuable compounds.

Carotenoids represent a large group of structurally diverse pigments synthesized by microorganisms and plants (Fig. 5). Carotenoids are used commercially as food colorants, feed supplements, nutraceuticals and for cosmetic and pharmaceutical purposes. Of the more

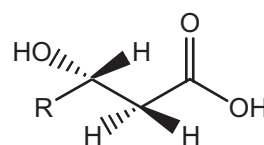


Fig. 4. *R*-3-HFA.

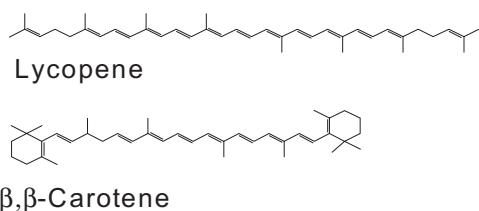


Fig. 5. Examples of carotenoid structures.

than 600 different carotenoids identified in nature, only a small number can be synthesized in useful quantities by chemical synthesis, extraction from their natural sources or microbial fermentation (reviewed in Lee and Schmidt-Dannert, 2002).

Over the last decade genes encoding the enzymes of the carotenoid biosynthetic pathways leading to carotenoids with C30, C40 or C50 carbon backbones have been cloned from microorganisms and plants. Biosynthesis of these pigments in non-carotenogenic hosts such as *Escherichia coli* or *Yarrowia* requires first the extension of its isoprenoid pathway with a gene encoding geranylgeranyl diphosphate synthase (CrtE), which catalyses head-to-tail condensation of an additional C5 isoprenoid unit to the general isoprenoid precursor farnesyl diphosphate (C15) to yield geranylgeranyl diphosphate (C20, GGDP). An additional recombinant enzyme, phytoene synthase (CrtB), is then required for the head-to-head condensation of two GGDP molecules to the colourless C40 carotenoid phytoene. To obtain coloured carotenoids in *E. coli*, a phytoene desaturase (CrtI) needs to be coexpressed to synthesize the orange-red acyclic carotenoid lycopene. Extension of this core pathway with known enzymes that catalyse cyclization, glucosylation and diverse oxygenation reactions allows the production of an array of acyclic and cyclic carotenoids and xanthophylls in *E. coli*. Production of C30 carotenoids occurs from FPP via a head-to-head condensation reaction catalysed by CrtM and desaturation of the resulting dehydrosqualene by CrN to diaponeurosporene or diapolyycopene. Expression of these enzymes in the host *E. coli* facilitated their characterization and allowed the recombinant biosynthesis of diverse cyclic and acyclic carotenoids in *E. coli* (Schmidt-Dannert *et al.*, 2000). Dr Schmidt-Dannert's group at the University of Minnesota has extensively explored combinatorial, evolutionary and genomic strategies for the production of diverse carotenoid structures and carotenoid cleavage compounds in engineered microbial hosts (Schmidt-Dannert, 2001; Mijts *et al.*, 2004; Marasco *et al.*, 2006). Many of these new carotenoid structures have valuable pigment and antioxidant properties. However, despite successes in creating these diverse recombinant carotenoid pathways, there is a pressing need to find ways of

producing carotenoid structures (most cannot be chemically synthesized) in sufficient quantities for commercial applications.

Polyenic polymers are new types of specialized, conducting polymers (Fig. 6) – also referred to as electronic plastics – have been developed for applications in an emerging class of 'plastic' opto-electronic devices such as lasers, ultra fast image processors, thin-film transistors, highly sensitive plastic photodiodes and photodiode arrays and all-polymer integrated circuits. These new polymers, like other commonly used plastics, are chemically synthesized from petroleum-derived building blocks. Biological routes to functional materials independent petroleum-based economy could instead be described as a biorefinery process in which biomass components are converted into high-value compounds.

Nature produces an array of stable conducting organic molecules designed and optimized to carry out electron transport and light-harvesting functions, the latter via photoinduced electron transfer. The largest class of molecules with delocalized electron systems – carotenoids – is synthesized from polycondensated isoprenoid building blocks. The conjugated polyisoprenoids resemble the first chemically synthesized electronic polymer polyacetylene, but contain additional methyl-groups along the chain and are in an all-*trans* conformation, which exert a stabilizing effect on the molecule and increases electrical conductivity. Dr Schmidt-Dannert's group has developed a unique biosynthetic platform for the production of end-group functionalized polyenic building blocks in metabolically engineered *E. coli* cells (Mijts *et al.*, 2005).

Microbial genome mining for novel biosynthetic enzymes together with combinatorial biosynthesis and *in vitro* evolution of biosynthetic enzymes was used to engineer *E. coli* strains that produce fully conjugated isoprenoid chains containing carboxylic acid, aldehyde or alcohol functions at both terminal ends. The produced conjugated and oxygenated isoprenoid chains (C30 and C40 carotenoids) are extremely stable (in fact, the compounds appear self-assemble and their unusual stability may be due to chain stacking) and represent ideal building blocks for the synthesis of conducting polymers. Their light-absorbing properties make them ideally suited for photoinduced electron transfer, a much-desired property of electronic plastics.

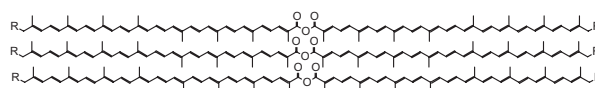


Fig. 6. Proposed structure of stacked polyenic carotenoid ester chains.

LipoYeasts project

The project's concept is illustrated on Fig. 7. The LipoYeasts project combines biosynthetic potential of hydrocarbonoclastic and other hydrocarbon and lipid-degrading bacteria of various lipid-contaminated environments with the lipid-mobilizing potential of the oleaginous yeast *Y. lipolytica* to produce valuable lipid-derived by-products, such as poly- and wax esters, HFAs, carotenoids and carotenoid esters. For this purpose, the lipid metabolism of *Yarrowia* is to be adjusted in such a way, that it creates a high pool of lipid-derived compounds, precursors of high-value products. The main metabolic routes to be optimized are β - and ω -oxidations and lipid accumulation. More specifically, we will concentrate on the construction of strains with reduced capacity in the storage of fatty acid into lipid bodies and with reduced capacity to degrade fatty acids by either β - or ω -oxidation, as shown in Fig. 7.

To develop *Y. lipolytica* into a fermentation platform for the conversion of lipid feed stocks into commercially interesting products, we propose the following complementary focus areas (WPs) of research and development: (i) engineering of metabolic precursor pools in *Y. lipolytica* for the production of value added products from lipids, (ii) conversion of metabolic precursor pools in *Yarrowia* to value-added products by overexpressing heterologous biosynthetic enzymes, (iii) discovery and characterization of novel aliphatic enzyme activities by metagenomic screening of marine hydrocarbonoclastic microbial communities, (iv) pilot process development and identification of commercial applications, and (v) project coordination and IPR management.

Already available for this project are expression vectors and several deletion strains of *Yarrowia* as well as a

collection of genes encoding enzymes for the production of WEs, 3-HFAs, PHAs and carotenoids. We can therefore immediately begin with WP1 to improve lipid precursor pools in *Yarrowia* and implementation of the proposed heterologous biosynthetic capabilities into *Yarrowia* (WP2). Parallel to these efforts we will add new enzyme functions identified in metagenomic libraries to our toolbox of lipid-modifying enzymes (WP3) for expression in *Yarrowia*. Once the first recombinant yeast producer strains have been generated, pilot processes for mid-scale production will be developed and applications for produced compounds identified.

Conclusion

Current imbalance between chemical technologies and biotechnologies to make use of bulk fats and oils is addressed by this collaborative project. It aims to create new possibilities to convert lipids into valuable products by using the oleaginous yeast *Y. lipolytica* as a lipid-mobilizing platform. This research will also hopefully bridge the gap between enzyme discovery studies based on the enormous amount of genomic information generated via genome sequencing projects on one hand and availability of easily genetically modifiable and well-studied lipid-mobilizing yeast on the other hand. By combining all these valuable and rather separated experiences together, we plan to make the practical use of this unique combination of available information and strains, by constructing yeast cell factories for the production of different high-value industrial compounds. Thus we hope to expand the knowledge: (i) in yeast metabolism by studying fluxes of fatty acids intermediates, (ii) of recently discovered but not fully characterized enzymes derived

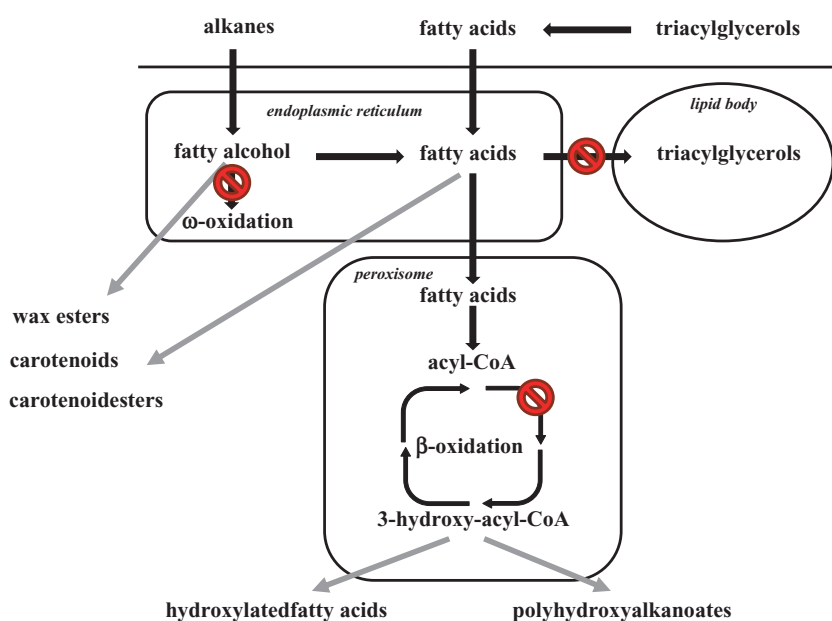


Fig. 7. Metabolic engineering of *Yarrowia lipolytica* for production of added-value products. Lipids such as triacylglycerols (TAGs), fatty acids and n-alkanes are used as carbon sources. They get hydrolysed (TAGs) extracellularly and get transported into the cell, where fatty acids get either stored as TAGs in lipid bodies or get further oxidized in ω - or β -oxidation cycles. Storage of fatty acids as TAGs will be blocked. β -Oxidation cycle provides precursors for hydroxylated fatty acids (HFAs) and polyhydroxyalkanoates (PHAs). Modifications of Pox1–6 genes catalysing the first step of β -oxidation will be explored for modulating monomer composition of the produced HFAs and PHAs. Finally, the ω -oxidation will be also blocked to avoid the production of undesired dicarboxylic fatty acids. The resulted pool of fatty acids and fatty acid alcohols will be used for the production of WEs, carotenoids or carotenoid esters by expressing the corresponding bacterial biosynthetic genes.

from hydrocarbonoclastic bacteria and to understand their function by their heterologous expression in *Yarrowia*, (iii) in enzyme mining and in establishment and improvement of screening protocols applied to metagenomes, (iv) in new enzymes and their unique properties, and (v) in novel applicational aspects of the produced compounds. The consortium collectively has all tools and expertise at their disposal to meet this goal via close interaction between the partners. The progress of the work will be posted on the consortium webpage (<http://www.lipoyeasts.ugent.be/>) inviting a wide range of feedback from the scientific community.

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