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REVIEW

Yeast Engineering for Antioxidant Production

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Abstract—This article reviews biosynthesis of the valuable fat-soluble compounds with antioxidant activity, in particular vitamin E isomers and carotenoids, in yeast cells. Main genetic engineering approaches to increase microbial production of these substances are described. The main innovative strategies for subcellular separation of synthesis, storage, and recovery of lipophilic compounds are discussed, and examples of cell morphology engineering importance of are shown.

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INTRODUCTION

Natural antioxidants, such as phenolic compounds, carotenoids, vitamins, and microelements, are found in nature mainly in plants. Fat-soluble antioxidants are strategically important molecules with a broad range of applications. The key antioxidants include such compounds as molecules of vitamin E (tocopherol and tocotrienols), carotenoids (β-carotenes, lycopene, lutein, astaxanthin). These compounds actively protect lipid structures against peroxidation, participate in regulation of signaling pathway, and are capable to reduce development of cardiovascular diseases, atherosclerosis, neurodegenerative processes, metabolic syndrome, allergy [1]. They also improve immune functions [2], exhibit anti-diabetes [3] and anti-inflammatory functions [4].

These compounds are widely used in food industry as preservatives, in cosmetics industry as stabilizers, as well as in feed industry [5-12]. For example, vitamin E plays a critical role in maintenance of health in farm animals providing resistance against oxidation, immunomodulation, preservation of fertility [13], and carotenoids in aquaculture not only provide antioxidant defense, but also facilitate desirable fish pigmentation [9, 12]. Due to unique functions of antioxidants, global demand for their use in everyday life increases, which attract interest of researchers and technologists to the development of effective methods of synthesis, bioproduction, and isolation of biologically active fat-soluble compounds of natural origin [14].

Traditional methods for isolation of biologically active compounds from plant raw materials are not very effective at present due to the high cost of extraction requiring costly processing methods, geographic factors, and dependence on seasons. In particular, approximately 2 g of mixture of α- and β-carotene could be produced from 50 kg of carrots [15]. And extraction of astaxanthin from natural sources (microalgae) could cost as high as US $7000 per kg in comparison with US $1000 per kg in the case of chemical synthesis [16].

Chemical synthesis, despite the low cost, often results in formation of a mixture of stereoisomers and xenobiotics that do not exist in nature. For example, the main part of the commercial astaxanthin is currently synthesized using a double Wittig reaction [5]. As a result, a racemic mixture of stereoisomers (3S,3′S), (3R,3′S), and (3R,3′R) is produced with an approximate ratio of 1:2:1 [17]. Such synthetic astaxanthin is used predominantly as pigment

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supplement in aquaculture feed to enhance coloring of such fishes as salmon, shrimp, crustaceans, and decorative fishes [18]. However, despite the absence of convincing evidence confirming different biological activity of each isomer [19], the chemically synthesized astaxanthin was not approved for human consumption due to the health problems associated with the presence of toxic intermediate and side products [20]. Chemical synthesis of vitamin E, which is rather complex, includes multistep alkene reaction under high pressure, and, hence, imposes high requirements in terms of equipment and safety measures, also produces a racemic mixture of isomers [21]. Commercial synthesis of pure isomers of tocopherols and tocotrienols has not been developed yet due to complexity of the process, low yield, and insufficient purity [22].

Explosive development of metabolic engineering and synthetic biology resulted in emergence of microbial factories, which now play a key role in scalable, controlled, sustainable, and economically effective bioproduction of valuable compounds [21]. Microbial cells provide the possibility for production of large quantities of biologically active compounds with minimal effect on the environment.

However, the issues of maintenance of intracellular homeostasis after introduction of heterologous synthetic pathways, decrease of the internal flows to competing reactions, optimization of cultivation conditions and scalability of fermentation processes, as well as development of the processes of isolation and purification of the target product remain not fully resolved [23].

The number of biotechnological processes using yeast cells as producers has increased in recent decades [24-29]. Yeasts offer some advantages in comparison with other microorganisms: they have large cells, which are capable of accumulating high biomass within rather short period of time, the yeast-based processes are easily scalable, and these cells are resistant to various stress factors and allow using a broad range of carbon sources including industrial waste and complex substrates. In this review we present recent advances in development of yeast-based microorganisms-producers for production of two main groups of fat-soluble antioxidants such as carotenoids and compounds of the vitamin E group.

BIOSYNTHESIS OF VITAMIN E

Vitamin E is a common name for tocopherols and tocotrienols, which are fat-soluble vitamins essential for humans [30]. Vitamin E consists of eight different molecules: α-, β-, γ-, and δ-tocopherols; as well as α-, β-, γ-, and δ-tocotrienols. Vitamin E molecules protect cells against oxidative stress by decreasing intracellular reactive oxygen species (ROS) and enhancing antioxidant defense. This property is especially valuable in the treatment of cells damaged in osteoporosis, neurodegenerative diseases, and aging [31]. It was shown in experimental studies that tocotrienols exhibit more pronounced antioxidant properties in comparison with tocopherol due to their structure and better distribution in lipid layers of cellular membranes. Unsaturated chain in tocotrienols facilitates effective penetration into the tissues with saturated fat layers, such as brain and liver [32]. It was also shown in the studies that δ-tocotrienol enhances regenerative potential of bone marrow stem cells, thus facilitating wound healing [33]. Tocotrienols also exhibit anticancer properties inhibiting tumor cell growth by inducing apoptosis and paraptosis and disrupting pro-tumor signals; and δ-tocotrienol exhibits enhanced activity in combination with traditional chemotherapy in pre-clinical studies [34]. In addition, molecules of vitamin E are used in the modern methods of drug delivery due to high lipophilicity of these molecules, including in composition of nanoemulsions and polymeric carriers, in order to improve bioavailability and targeted therapeutic actions of different drugs [35].

In nature biosynthesis of vitamin E compounds occurs predominantly in plants, algae, and certain cyanobacteria, which are photosynthesizing organisms [36]. In plants vitamin E biosynthesis occurs at the inner envelope of plastids through combination of two main pathways (Fig. 1). The biosynthetic pathway involves two main precursors: homogentisic acid (HGA) from the shikimate pathway, which is condensed with geranylgeranyl pyrophosphate (GGPP) from the mevalonate (MVA) pathway in the case of tocotrienol synthesis, or with phytyl pyrophosphate (PhPP), also from the MVA pathway in the case of tocopherol synthesis [37]. As a result of condensation reaction catalyzed either by homogentisate geranylgeranyl transferase (HGGT), or homogentisate phytyltransferase (HPT), 2-methyl-6-geranylgeranyl benzoquinone (MGGBQ) is formed. Next, MGGBQ is subjected to methylation and/or cyclization under the action of three main enzymes, 2-methyl-6-phytyl benzoquinone methyltransferase (MPBQMT), tocopherol cyclase (TC), and γ-tocopherol methyltransferase (γ-TMT). Specific presence and sequence of these enzymatic reactions result in the synthesis of δ, γ, α, and β forms of tocopherols and tocotrienols.

BIOSYNTHESIS OF CAROTENOIDS

Carotenoids comprise a diverse group of natural compounds of the class of tetraterpenoids containing isoprene polymers with 40 carbon atoms. Carotenoids

play important functions in plants, animals, and microorganisms, including body pigmentation, biocommunication, synthesis of vitamin A, and also exhibit strong antioxidant properties. It has been forecasted that the market for carotenoids would grow from 1.5 billion US dollars in 2019 to 2 billion US dollars by 2026 [38]. Carotenoids have a condensed structure, which implies that they absorb blue and green light, and are colored from yellow to red. At present more than 1100 carotenoids have been described [39]. Carotenoids with the highest antioxidant properties include:

1) Astaxanthin, which has a unique ability to neutralize reactive oxygen species and free radicals, exhibits activity 6000-fold higher than activity of vitamin C, 200-fold higher than activity of tea polyphenols, and 17-fold higher than activity of the extract of grape seeds [40]. Astaxanthin protects against oxidative stress, reduces inflammation, and maintains skin health and health of cardio-vascular system.
2) Lycopene effectively eliminates free radicals and protects cells against damages. It also decelerates aging processes, decreases risk of cancer development, and improves skin state [41].
3) β-Carotene is transformed in an organism into retinol (provitamin A), which is important for development, growth, and vision. Among all carotenoids, this compound is the highest source of vitamin A [15]. The US Food and Drug Administration (FDA) approved the use of β-carotene as a food supplement and in composition of baby formula as a source of vitamin A.
4) Canthaxanthin that exhibits strong antioxidant effects eliminating free radicals and stimulates activities of catalase and superoxide dismutase facilitates protection of cellular membranes against peroxidation and reduces inflammation [42].

Biosynthesis of all carotenoids is based on sequential condensation of the 5-carbon monomer of isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (Fig. 1) synthesized in yeast via the mevalonate pathway (MVA) [43, 44]. IPP and DMAPP are subjected to condensation with formation of 10-, 15-, and 20-carbon molecules of geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP) in two reactions catalyzed by the enzymes farnesyl pyrophosphate synthase and geranylgeranyl pyrophosphate synthase, respectively [45]. Next, the enzyme phytoene synthase catalyzes condensation reaction of two GGPP molecules producing a phytoene molecule. Phytoene is transformed into lycopene in the dehydrogenase reaction catalyzed by phytoene dehydrogenase. After that, β-carotene is synthesized from lycopene in the reaction catalyzed by lycopene β-cyclase [46]. It is known that both reactions of phytoene and β-carotene synthesis could be catalyzed by one bifunctional enzyme phytoene synthase/lycopene β-cyclase [47]. Canthaxanthin is synthesized from β-carotene by introducing keto-groups with the help of the β-carotene ketolase enzyme [27]. Astaxanthin is synthesized from canthaxanthin by introducing hydroxy groups with the help of the β-carotene-3-hydroxylase [48], or from β-carotene via oxidation of β-carotene in the reaction catalyzed by astaxanthin synthase operating together with the cytochrome P450 reductase [24].

SOLUTIONS FOR THE UNIQUE CHALLENGES DURING MICROBIOLOGICAL SYNTHESIS OF LIPOPHILIC COMPOUNDS

The production process of carotenoids, tocopherols, and tocotrienols in yeast cells could be divided into two modules. The first module includes production of native precursors synthesized according to the mevalonate pathway (for carotenoids) or synthesized in two pathways – mevalonate pathway and shikimate pathway (for vitamin E). Further synthesis proceeds by the non-native pathway that involves heterologous enzymes from different organisms [26, 23, 49]. However, simple integration of the genes of heterologous enzymes under control of strong promoters does not guarantee a good level of synthesis of the final compound. In order to maintain stable and balanced functioning of the yeast cell-producers it is necessary to increase activity of native metabolic pathways together with fine optimization of expression of the heterologous genes. Moreover, lipophilic compounds are poorly transported across the subcellular and cellular membranes due to their hydrophobic nature and could exhibit toxic effect if accumulated in the cells thus interfering with further cell growth.

Production of fat-soluble antioxidants in yeast cells became possible due to the introduction of complex strategies of metabolic engineering, improved methods of fermentation, and new systems controlling intracellular processes. Significant increase of the yields of tocopherol, tocotrienols, and carotenoids reported in the emerging papers as well as increase of production efficiency make yeast cells an attractive platform for commercial production of antioxidants and similar compounds.

Main studies and advances in biosynthesis of tocopherols and tocotrienols, as well as of β-carotene, lycopene, canthaxanthin, and astaxanthin, will be discussed further in this review. Main genetic engineering approaches will be described, and innovative strategies for enhancing production of these compounds in yeast will be presented. Summary of the realized strategies and corresponding fermentation results are shown in Table 1.

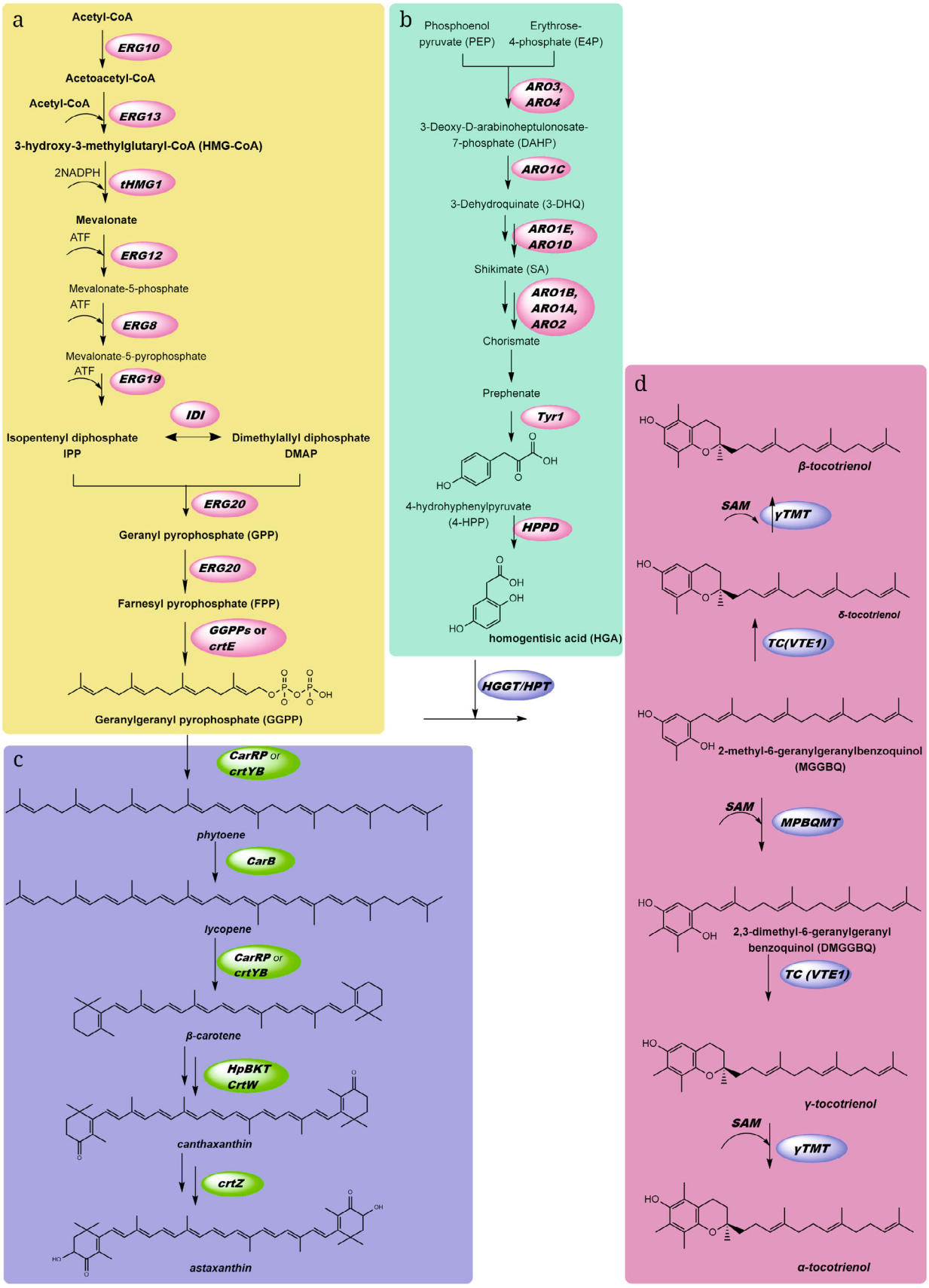

Fig. 1. Pathway of carotenoids and vitamin E biosynthesis in yeasts. a) Scheme of mevalonatepathway; b) scheme of shikimate pathway; c) carotenoid synthesis pathway: lycopene, β-carotene, canthaxanthin, astaxanthin; d) tocotrienol synthesis pathway.

Table 1. Brief description of genetic modifications of yeast cells that improved yields of carotenoids, tocotrienols, and tocopherols

Yeast-producers	Genetic modifications	Production, cultivation method	References
	δ-tocotrienol		
S. cerevisiae	HPPD (HPD) of *Pseudomonas putida*, HGGT/HPT of *Synechocystis* sp., VTE1 of *Arabidopsis thaliana*, ↑GGPP: tHMG1, GGPPsa of *Sulfolobus acidocaldarius*	4.10 mg/l (2-l fermenter)	[50]
S. cerevisiae	HPPD of *A. thaliana*, HGGT of *Synechocystis* sp. (slr1736), VTE1 of *A. thaliana*; complex HPPD–SH3 ligand/SH3 domain–HGGT–GGGGS3–tTC; ↑GGPP: CrtE, FPSF112A; ↑HGA: ΔAro10, ΔAro3, △Pdc5, Aro4 (K229L), Aro7 (G141S), Tal1, Tyr1, Eno2, Aro2; expression of endogenous reporter Pdr11p	3.262 mg/l (flasks)	[26]
Y. lipolytica	HGGT/HPT of *Synechocystis* sp. and *Triticumae*., VTE1 of *A. thaliana* (VTE1); design of SyHPT, fusion SyHPT-GSG-AtVTE1; ↑GGPP: tHMG, GGPPs of *S. acidocaldarius* and *Xanth*.; ↑HGA: ARO4K221L, ARO7G139S, Aro1	467 mg/l (5-l fermenter)	[53]
	α, β, γ, δ-tocotrienols		
S. cerevisiae	HPPD, tMPBQMT, tγ-TMT, tVTE1 of *Arabidopsis thaliana*; SyHPT of *Synechocystis* sp.; tHMG1, crtE (GGPP) of *Xanth. dendrorhous*; ↑HGA:ΔAro10, Aro4(K229L), Aro7(G141S), TyrC, Tkl1	320 mg/l (5-l fermenter)	[51]
S. cerevisiae	ΔMOT3, ΔROX1 ↑GGPP: tHMG, CrtE(CrtE03M), POS5 (↑NADPH); expression of endogenous reporters Pdr11p, Yol075cp	82.68 mg/l (flasks)	[52]
	β-farnesene		
S. cerevisiae	↑GGPP: ERG8, 2× tHMG1, ERG12, MVD1, ERG10, IDI1, and ERG13; mutant synthase 5× bFS	791 mg/l (flasks)	[21]
	Lycopene		
S. cerevisiae	2× TmCrtE, PaCrtB, 2× BtCrtI, tHMG1, ADH2, SeACS, ALD6, PAH1, DGA1, ACC1S659A, S1157A, OLE1	2.4 g/l (7-l bioreactor)	[54]
	DwCrtE, DwCrtB, DwCrtI	10.1 mg/g of dry weight (flasks)	[55]
Y. lipolytica	XdCrtE, CarB, CarRPE78K, ERG12, tHMG1, ERG20, IDI, CK, IPK	17.6 g/l (3-l bioreactor)	[56]
	β-carotene		
S. cerevisiae	XdCrtE, XdCrtYB, XdCrtI, Δald6, SsXYL1, SsXYL2, ScXKS1; integrated xylose utilization pathway	772.8 mg/l (3-l bioreactor)	[50]
	XdCrtE, XdCrtYB, XdCrtI, YlLIP2, YlLIP7, YlLIP8; expression of lipases from *Y. lipolytica* for utilization of hydrophobic substrates	477.9 mg/l (flasks)	[57]

Table 1 (*cont.*)

Yeast-producers	Genetic modifications	Production, cultivation method	References
Y. lipolytica	2× GGS1, CarB, 5× CarRP, 3× tHMG1, Δpox3-6, IDI, ERG10, ERG12, ERG13	4.0 g/l (2-l bioreactor)	[58]
	GGS1, CarB, CarRP, HMG1, Δpox1-6, tgl4, DGA2, GPD1	6.5 g/l (5-l bioreactor)	[59]
	GGS1, 2× CarB, 3× CarRP, HMG1, Δpox2-3, ERG13	4.5 g/l (5-l bioreactor)	[46]
	GGS1, CarB, CarRP, Δpex10, ACBP ZWF1, ACC1, DGA1, ScSUC2, HXK1; saccharose utilization pathway introduced	625.8 mg/l (tubes)	[60]
	XdCrtE, CarB, $CarRP^{Y27R}$, ERG12, tHMG, ERG20, IDI, CK, IPK	39.5 g/l (3-l bioreactor)	[56]
	Complex CarRP-GGPPs7, CarB HMG1, ERG12, ERG20, Δpex10, ACBP ZWF1, ACC1, DGA1, ScSUC2, HXK1	149 mg/l (tubes)	[61]
	GGS1, fused CarRP-GGPPs7, fused CarRP-$ERG20^{F88C}$, CarRP, 2× CarB, HMG1, ERG12, ERG20, Δpex10, ACBP, ZWF1, ACC1, DGA1, ScSUC2, HXK1	2.4 g/l (3-l bioreactor)	[62]
	Canthaxanthin		
S. cerevisiae	2× CrtE03M, 2× XdCrtI, 4× XdCrtYB, SctHMG1, OBKTM29, $PM^{SeV\text{-}C}$-OBKTM29, PDR1, PDR3, GAL4M9; increased stress resistance	1.4 g/l (5-l bioreactor)	[27]
Y. lipolytica	GGS1, CarB, CarRP, BsCrtW	36.1 mg/l (flasks)	[63]
	GGS1, fused CarRP-GGPPs7, fused CarRP-$ERG20^{F88C}$, CarRP, 2× CarB, 3× HpBKT, HMG1, ERG12, ERG20, ACBP, ZWF1, ACC1, DGA1, ScSUC2, HXK1, SpHXK1, YHT1, YHT3, YHT4, POT1; expression of hexose transporters, effective saccharose utilization	1.8 g/l (3-l bioreactor, saccharose) 1.2 g/l (3-l bioreactor, hydrolysate of methanotrophs + molasses)	[64]
	Astaxantine		
S. cerevisiae	CrtE, CrtI, CrtYB, BTS1, BvCrtW, AaCrtZ, ScERG20, SctHMG1, ΔCSS1	217.9 mg/l (5-l bioreactor)	[65]
	CrtE03M, 2× CrtI, 3× CrtYB, 2× OCrtZM1, OBKTM29, tHMG1	446.4 mg/l (5-l bioreactor)	[66]
Y. lipolytica	XdCrtE, XdCrtYB, XdCrtI, PsCrtW, PaCrtZ, HMG1, ↓SQS	54.6 mg/l (culture plate)	[24]
	XdCrtE, SsGGPPS, XdCrtYB, XdCrtI, HpBKT, HpCrtZ, HMG1, ↓SQS	285 mg/l (1-l bioreactor)	[48]
	SaGGPPS, CarRP, CarB, fused PsCrtW-HpCrtZ-SKL, fused PsCrtW-HpCrtZ-oleosin, fused PsCrtW-HpCrtZ-KDEL, localization in peroxisome, endoplasmic reticulum, and lipid droplets	858 mg/l (3-l bioreactor)	[67]

Table 1 (*cont.*)

Yeast-producers	Genetic modifications	Production, cultivation method	References
Y. lipolytica	tHMG1, GGS1, CarRP, CarB, multiple copies of HpBKTRIDD, multiple copies of HpCrtZRIAD	3.3 g/l (5-l bioreactor)	[69]
	GGS1, fused CarRP-GGPPs7, fused CarRP-ERG20^{F88C}, CarRP, 2× CarB, 3× HpBKT, 3× HpCrtZ, HMG1, ERG12, ERG20, Δpex10, ACBP ZWF1, ACC1, DGA1, ScSUC2, HXK1	973 mg/l (3-l bioreactor, glucose + olive oil)	[62]
	2× GGPPSa, ERG20^{F88S}, CrtYB, 4× CarRP^{Y27R}, 4× CarB, multiple copies of HpCrtZ-RIAD and HpBKT-RIDD, multiple copies of HpcrtZ^{N183A}-RIAD and HpBKT^{V264D}-RIDD, multiple copies of HpBKT^{V264D}-RIDD-Oleosin, ↓SQS, 2× tHMG1, MVAE, MVAS, ERG12, 2× IDI DGA1	2.8 g/l (5-l bioreactor)	[69]

MAIN ACIEVEMENTS IN BIOTECHNOLOGY OF VITAMIN E PRODUCTION

Pathways of tocopherol biosynthesis in nature are observed in photosynthesizing organisms, such as plants and certain algae, but they are absent in yeasts. Hence, the endogenous mevalonate and shikimate pathways, which mediate synthesis of precursors (geranylgeranyl pyrophosphate and 4-HPP/homogentisic acid) are supplemented with the genes of heterologous enzymes required for complete synthesis of tocopherols and tocotrienols: 4-hydroxyphenylpyruvate dioxygenase (HPPD), HPT/HGGT, MPBQMT, TC, and γ-TMT [51, 53].

HPPD transforms 4-hydroxyphenyl pyruvate (4-HPP) into homogentisic acid (HGA), aromatic precursor of vitamin E; HPT/HGGT (homogentisate phytyl transferase/homogentisate geranylgeranyl transferase) catalyzes condensation of HGA with phytyl diphosphate (to produce tocopherols) or with geranylgeranyl diphosphate GGPP (to produce tocotrienols); MPBQMT (MPBQ methyltransferase) methylates condensed (prenylated) intermediate products in this pathway; TC (tocopherol cyclase) catalyzes cyclization of intermediate products with formation of tocopherol and tocotrienol rings; γ-TMT (gamma-tocopherol methyltransferase) performs further methylation of intermediate products of tocopherol or tocotrienol with formation of various isoforms.

The first successful *de novo* biosynthesis of δ-tocotrienol was realized in the cells of baker's yeast *Saccharomyces cerevisiae*. In their study Hong Sun [50] with colleagues in 2020 constructed heterologous biosynthetic pathway in *S. cerevisiae* by introduction of the genes from different species: HPPD (HPD) from *Pseudomonas putida*, HPT from *Synechocystis* sp., and VTE1 from *Arabidopsis thaliana.* To increase the amount of a key precursor, geranylgeranyl diphosphate (GGPP), the researchers overexpressed the supplementary genes tHMG1 (truncated gene 3-hydroxyl-3-methylglutaryl-CoA reductase) and GGPPs (geranylgeranyl diphosphate synthase) from *Sulfolobus acidocaldarius*. In the course of initial fermentations in glucose, the titer of delta-tocotrienol was 1.39 mg/l, which further was increased to 3.56 mg/l by optimizing fermentation medium using response surface methodology. Further improvement using fed-batch fermentation in a 2-l fermenter allowed increasing the titer to 4.10 mg/l.

In the next study [51] with *S. cerevisiae* yeast another strain-producer of δ-tocotrienol was constructed by integration of four plant genes HPPD, MPBQMT, γ-TMT, codon-optimized TC from *A. thaliana*, and one codon-optimized gene of HPT from cyanobacteria *Synechocystis* sp. into *S. cerevisiae*. The sequences of some proteins (MPBQMT, TC, and γ-TMT) were truncated by removing the plant transit peptides from the N-end to improve enzyme functioning in yeasts. Expression of GGPP via the mevalonate pathway (MVA) was enhanced by overexpression of tHMG1 and introduction of the GGPPs CrtE from *Xanthophyllomyces dendrorhous*; and ensuring of sufficient supply 4-HPP via cofactor enhancement resulted in the higher level of HGA synthesis. These modifications allowed improving the precursor formation via the mevalonate and shikimate pathways and eliminate metabolic bottlenecks thus increasing total yield of trienols. A new two-step system of temperature control was developed for the enzymatic synthesis induced by cold shock, which allowed increasing the yield to 7.6 mg/g of dry biomass and the titer of 320 mg/l in the 5-l fermenter. This control system optimizes the phases of cell growth and product

accumulation, which increases the overall efficiency of production. Temporal separation of the growth phase and production phase allowed to eliminate metabolic stress with preservation of high product yield.

The same research group from the Institute of Bioengineering, Hangzhou, published a new paper in 2022 [52], which provided a significant contribution to the development and simplification of the process of extraction and purification of tocotrienols by improving secretory production of tocotrienols in the modified *S. cerevisiae* strain. This approach makes the following processes of purification and scaling-up production easier. First, the scientists optimized the heterologous pathway of tocotrienol synthesis by increasing supply of precursor through removal of the MOT3 gene encoding transcriptional repressor of hypoxic genes, which increased production of ergosterol for improving membrane fluidity and its permeation capacity [70], and of the ROX1 gene, transcriptional repressor, inactivation of which increased production of carotenoids [71]. The mevalonate pathway (MVA) was enhanced by introduction of additional copy of the *tHMG* gene encoding the truncated HMG-CoA reductase, additional copy of NADPH kinase POS5 [55] that increases the NADPH/NADP ratio required for functioning of the additional tHMG, as well as overexpression of the mutant GGPP synthase (CrtE03M) [25]. Next, excretion of tocotrienols into the culture medium was realized by the two-phase fermentation in 50-ml flasks with rich medium supplemented with olive oil as an extractant together with overexpression of endogenous PDR-transporters Pdr11p and Yol075cp. As a result, the yield of tocotrienols was increased to 25.57 mg/g of DCW with 73.66% secreted into the organic phase [52]. Isolation of tocotrienols as extracellular products simplifies significantly the following purification process and could serve as an example for the development of fermentation technology for production of other hydrophobic products.

Luyano Han [26] with colleagues created and optimized the pathway of *de novo* biosynthesis of one form of vitamin E – δ-tocotrienol, also in *S. cerevisiae*. In this study bottlenecks that limited the pathway flow were eliminated, and the key enzymes were combined into one module, which allowed to create a system of unidirectional substrate transfer and increase efficiency of the catalyzed reactions. The mevalonate pathway was enhanced via increase of production of GGPP by the heterologous GGPP-synthases (CrtE [55] and FPSF112A [72]), the enzymes of δ-tocotrienol synthesis pathway were optimized, and tVTE1 [51, 73] together with HGGT were anchored in endoplasmic reticulum [53]. Overexpression of the endogenous transporter Pdr11 and two-phase extractive fermentation was used for production of δ-tocotrienol providing δ-tocotrienol yield of 3262.2 μg/l.

Another interesting study was published in 2022 in which Ye et al. [21] suggesting a new effective process for biosynthesis of the vitamin E precursor – farnesene, in yeast. Next, farnesene is transformed into isophytol with 92% efficiency, which further is transformed into α-tocopherol in the condensation reaction with trimethylhydroxyphenone. Based on the process developed in this study a factory was built in China in 2017, which produces 300,000 ton of vitamin E annually. The method is based on optimization of the mevalonate pathway for excessive production of β-farnesene in the *S. cerevisiae* cells and engineering of β-farnesene synthase (bFS) of *Artemisia annua.* Engineering of bFS involved introduction of random mutations using a special polymerase during PCR. The high-throughput screening allowed to identify the best variant of the synthase with 5 mutations, BFS45 (F11S, M35T, T319S, I434T, I460V). To enhance the flow in the mevalonate pathway, majority of the genes in this pathway were overexpressed (ERG8, tHMG1, ERG12, MVD1, ERG10, IDI1, and ERG13). In order to balance the upstream (MVA pathway) and downstream (farnesene-synthase) pathways of farnesene synthesis, the number of copies of *tHMG1* (2 copies) and *bFS* mutant (5 copies) were optimized. The final strain allowed production of 791 mg/l of β-farnesene during cultivation in flasks with 50 ml of rich medium [21].

In one of the recent studies by Xiang et al. [53], the *Yarrowia lipolytica* strain was designed that could be used for *de novo* biosynthesis of δ-tocotrienol by integration of complete biosynthetic pathways and increase of precursor supply. The shikimate pathway was enhanced by overexpression of the mutant genes $ARO4^{K221L}$ and $ARO7^{G139S}$ [74], the MVA pathway was enhanced by overexpression of the key genes, as well as by combined expression of the genes of GGPP-synthase from two different sources, *Sulfolobus acidocaldarius* and *Xanthophyllomyces dendrorhous*. To increase catalytic activity of the enzymes, the strategy of fusion of the genes encoding key enzymes SyHPT and AtVTE1 was employed using linkers forming substrate catalytic channels, as well as semi-rational design to improve activity of the SyHPT enzyme, and integration of multiple copies of the genes. These approaches allowed to increase the titer of δ-tocotrienol to approximately 467 mg/l in the fed-batch reactor, which is the highest yield of microbial synthesis of tocotrienols reported in the literature so far [53].

RECENT ACHIEVEMENTS IN BIOTECHNOLOGY OF CAROTENOID PRODUCTION

To construct lycopene producer in *S. cerevisiae*, yeast the following genes were expressed: Tm*CrtE*

encoding geranylgeranyl pyrophosphate synthase of *Taxus mairei*, Pa*CrtB* encoding phytoene synthase of *Pantoea agglomerans* and Bt*CrtI* encoding phytoene desaturase of *Blakeslea trispora*. Lycopene production in a 7-liter bioreactor was 2.37 g/l and 73.3 mg/l of dry weight [54].

The phenomenon of substrate inhibition of lycopene β-cyclase was identified as a key limiting factor of carotenoid biosynthesis in the *Y. lipolytica* yeast [57]. During optimization of lycopene synthesis pathway and its accumulation in the cell, the attempts to increase expression of lycopene cyclase by increasing copy number of its gene or introduction of lycopene cyclases from other organisms did not result in the increase of β-carotene synthesis. The problem of lycopene accumulation remained unsolved, which implied that the level of the lycopene cyclase protein is not the limiting factor in this case. As a result, two variants of mutations in the CarRP (phytoene synthase/lycopene β-cyclase) gene encoding a bifunctional enzyme phytoene synthase/lycopene β-cyclase of *Mucor circinelloides*, $CarRP^{Y27R}$, and $CarRP^{E78K}$, were suggested. The $CarRP^{Y27R}$ mutation eliminated substrate inhibition and provided a high level of β-carotene production, while the $CarRP^{E78K}$ mutation resulted in accumulation of lycopene by stopping synthesis at this stage. With the help of this strategy together with introduction of the *XdCrtE* and *CarB* genes encoding enzymes geranylgeranyl pyrophosphate synthase of *Xanthophyllomyces dendrorhous* and phytoene dehydrogenase of *M. circinelloides,* respectively, as well as also enhancing overexpression of the genes of mevalonate pathway and supplementation of the pools of IPP and DMAPP from isopentanol is was possible to reach in the 3-l bioreactor a record level of lycopene titer of 17.6 g/l, as well as the highest among the described in the literature production of β-carotene – 39.5 g/l [56].

Synthesis of β-carotene in *S. cerevisiae* yeasts was realized through heterologous expression of the *XdCrtYB*, *XdCrtI*, and *XdCrtE* genes encoding enzymes from *X. dendrorhous* – bifunctional phytoene synthase/lycopene cyclase, phytoene desaturase, and geranylgeranyl diphosphate synthase, respectively [75]. However, the producers based on *S. cerevisiae* accumulated significantly lower amounts of β-carotene (up to 772.8 mg/l, Table 1).

The studies by the R. Ledesma-Amaro research group [59] also contributed to understanding the process of designing the β-carotene producer based on *Y. lipolytica*. It was shown in the studies that the fat-soluble β-carotene is produced in higher amounts in the yeasts capable of overaccumulation of lipids that form the so-called lipid bodies. Expression of the *Hmg1*, *Ggs1*, *CarRP*, and *CarB* genes encoding 3-hydroxy-3-methylglutaryl-CoA reductase (HMG1) and geranylgeranyl pyrophosphate synthase (GGS1) from *Y. lipolytica*, as well as the bifunctional enzyme phytoene synthase/lycopene β-cylase (CarRP) and phytoene dehydrogenase (CarB) from *Mucor circinelloides* in the strain of *Y. lipolytica* capable of accumulating lipids allowed producing β-carotene in the amounts of 6.5 g/l (90 mg/g of dry weight) with simultaneous synthesis of 42.6 g/l of lipids.

The following approaches were used to design a microbial cell factory for bioproduction of canthaxanthin. A mutant variant of β-carotene ketolase of *H. pluvialis OBKTM29* (with H165R, V264D, F298Y, M1T, N188D, L271R mutations) was expressed in the *S. cerevisiae* yeasts producing β-carotene [27]. To increase stress resistance of the yeast cells, the pleotropic regulators of drug resistance Pdr1 and Pdr3 were simultaneously activated. However, fermentation of this recombinant organism in a bioreactor was accompanied by the slow-down of growth and premature cessation of production. To address this problem the scientists suggested the system of two-phase fermentation induced by cold shock. This strategy ensured production of canthaxanthin with titer 1.436 g/l during 260-h fermentation in a 5-l bioreactor.

A new approach to canthaxanthin production was demonstrated in the recent study, which was based on bioconversion of the greenhouse gas methane into canthaxanthin using *Methylococcus capsulatus (Bath)* biomass and recombinant yeast *Yarrowia lipolytica* [64]. For this purpose, the *Y. lipolytica* strain producing β-carotene was modified via expression of more than ten genes of the canthaxanthin synthesis pathway (HpBKT – β-carotene ketolase from *Haematococcus pluvialis*), enhancement of acetyl-CoA flow, and increase of hexose catabolism. In particular, the genes of invertase ScSUC2 of *S. cerevisiae*, hexokinase SpHXK1 from *Schizosaccharomyces pombe*, and hexose transporters YHT1, YHT3, and YHT4 were introduced to accelerate sugar consumption. The developed strain allowed production of canthaxanthin at the titer 1.8 g/l already after 120 h of fermentation with saccharose as a carbon source. In addition, a fermentation medium was developed based on the biomass of methanotrophic bacterium *M. capsulatus (Bath)*. Scaling-up in a 3-l bioreactor with the new defined medium allowed to achieve the canthaxanthin titer of 1.4 g/l with saccharose, and 1.2 g/l, when saccharose was replaced with molasses, byproduct of sugar industry [64].

The best performance indicators of astaxanthin production in *S. cerevisiae* were reported by Min Li et al.; using the two-phase fermentation together with the thermo-sensitive system GAL4M9 the titer of 446.4 mg/l was achieved [66]. For this purpose, the following genes were introduced: *tHMG1*, mutant

versions of β-carotene ketolase OBKTM29 and β-carotene hydroxylase OCrtZM1 (*HpCrtZ* with L288R mutation) from *H. pluvialis*, as well as CrtE03M (*CrtE* with C81T, A908G mutations) from *X. dendrorhous* encoding geranylgeranyl diphosphate synthase, and *CrtYB* from the same species encoding the bifunctional phytoene synthase/lycopene cyclase.

The *Y. lipolytica* yeasts also are being actively investigated as a platform for astaxanthin production. In the study by Ma et al. [67], the initial strain was a variant with enhanced β-carotene synthesis produced by integration of the *SaGGPPS* gene of *Sulfolobus acidocaldarius*, as well as CarRP and CarB genes from *M. circinelloides*. Next three different genes were introduced encoding β-carotene ketolase and hydroxylase, and the obtained combinations including PsCrtW and HpCrtZ from *Paracoccus* sp. were tested, which allowed to obtain better titers of astaxanthin. Development of the enzymatic hybrid PsCrtW-HpCrtZ and targeting this complex to the cellular compartments with the help of signaling sequences allowed to demonstrate yield of astaxanthin of 858 mg/l.

Zhu et al. [69] also modified the *Y. lipolytica* strain to produce a high level of astaxanthin. First, the genes of β-carotene ketolase (*HpCrtZ*) and β-carotene hydroxylase (*HpBKT*) were selected that demonstrated the highest activity in transformation of β-carotene into astaxanthin in *Y. lipolytica*. Next, the modular assembly of the enzymes HpCrtZ and HpBKT with the RIDD/RIAD peptides was performed [69]. The peptides RIDD and RIAD are short hydrophobic polypeptide motifs that originate form the proteins regulators of signaling pathway, which are used for creation of specific protein-protein interactions, for example, they are used for effective fusion of the enzymes thus facilitating their coordinated functioning and improved metabolic flow in the cell [76]. The authors integrated 20 copies of each of the modified genes (*HpBKT-RIDD* and *HpCrtZ-RIAD*), together with the expression of the genes of β-carotene synthesis pathway (*GGS1*, *CarRP*, *CarB*) and mevalonate pathway (*tHMG1*). The use of this approach allowed to achieve the astaxanthin titer of 3.3 g/l (26.5 mg/g of dry weight) after 264-h fermentation in the 5-l bioreactor [69], which is the highest level described in the scientific literature.

MAIN STRATEGIES IN METABOLIC ENGINEERING FOR PRODUCTION OF VITAMIN E AND CAROTENOIDS

Enhancement of synthesis of the shikimate pathway precursors. Biosynthesis of aromatic amino acids in *S. cerevisiae* is strongly suppressed by phenylalanine and tyrosine via the feedback mechanism. It was shown that this inhibition could be overcome by introduction of the allele of tyrosine-insensitive gene *ARO4* (*ARO4*^{G226S}) [77, 78] in combination with deletion of another allele of 3-deoxy-D-arabinose-heptulosonate-7-phosphate synthase (DAHP) (*ARO3*), which results in 4-fold increase of flow in the pathway of aromatic amino acid synthesis. In addition, introduction of the mutant variant of chorismate mutase (*Aro7G141S*) [79] together with codon-optimized tyrosine-insensitive cyclohexadienyl dehydrogenase (*TyrC*) from *Zymomonas mobilis* [80] also facilitates reduction of tyrosine inhibition via the feedback mechanism. Overexpression of transketolase Tkl1 increases pool of erythrose-4-phosphate, which is an important precursor in the synthesis of aromatic amino acids [81]. It was shown in the studies [51, 82] that all these modifications resulted in the 2.9-fold increase of HGA synthesis. The optimized strain produced 3.6 mg/g of dry weight of tocotrienols in the absence of exogenous tyrosine, which is 1.4-fold higher in comparison with the conditions with added tyrosine [51].

The similar strategy for the increase of HGA level was used by Han et al. [26], which included inactivation of the genes of phenylpyruvate decarboxylase *Aro10*, 3-deoxy-7-phosphoheptulonate synthase *Aro3* and *Pdc5*, expression of the mutants *Aro4*^{K229L} and *Aro7*^{G141S}, as well as overexpression of the genes *Tal1*, phosphopyruvate hydratase *Eno2*, chorismate synthase *Aro2*, and cyclohexadienyl dehydrogenase *Tyr1*. As a result, HGA formation increased 5.1-fold; the δ-tocotrienol titer in the YT28 strain was 796.1 μg/l without exogenous tyrosine.

The following strategy for increase of HGA synthesis (important precursor in the pathway of tocopherol and tocotrienol synthesis) was suggested [62]: suppression of the competing pathway of tyrosine synthesis followed by enhancement of the *de novo* HGA synthesis. 4-Hydroxyphenylpyruvate is a common precursor for synthesis of HGA and tyrosine, hence, the genes of aromatic aminotransferases *ARO8*, *ARO9*, and *AAT2*, responsible for transfer of 4-hydroxyphenylpyruvate to the synthesis phenylalanine and tyrosine were deleted, while the mutants *ARO4(K229L)* and *ARO7(G141S)* resistant to the tyrosine and phenylalanine feedback and the HPD1 gene encoding 4-hydroxyphenylpyruvate dioxygenase from *Y. lipolytica* were integrated for enhancement of the *de novo* HGA synthesis. The conducted engineering of the synthesis pathway resulted in the significant accumulation of HGA and its polymerization into pyomelanin [62, 83].

Optimization of mevalonate pathway. Optimization of mevalonate pathway usually is focused on the increase of the geranylgeranyl pyrophosphate (GGPP) supply using different approaches. This increase

of mevalonate supply is realized through overexpression of the truncated gene of 3-hydroxyl-3-methylglutaryl-CoA reductase (*tHMGR*) [25, 84, 85], gene of FPP-synthase (*ERG20*), and isopentenyl diphosphate isomerase (IDI) [86].

GGPP is synthesized in yeasts from IPP and DMAPP through geranyl diphosphate (GPP) and FPP. The mutant FPP-synthase (FPSF112A) can directly synthesize GGPP from IPP and DAMPP, in this way competition with FPP is reduced. In order to increase production of GGPP, Luyao Han with colleagues introduced a heterologous GGPP-synthase of *Taxus* × *media* and a mutant FPSF112A of *Gallus gallus*. As a result, the titer of δ-tocotrienol increased 5.5-fold [26].

Hong Sun [50] with colleagues in their study devoted to production of tocotrienol in *S. cerevisiae*, overexpressed the truncated gene of hydroxymethylglutaryl-CoA-reductase (*tHMGR)* and the gene of heterologous geranylgeranyl pyrophosphate synthase (*GGPPs*) from *Sulfolobus acidocaldarius* to enhance the mevalonate pathway, which resulted in the increase of delta-tocotrienol titer to 1.39 mg/l.

In addition to the basic changes associated with overexpression tHMG1 and integration of geranylgeranyl pyrophosphate synthase CrtE from *Xanthophyllomyces dendrorhous*, the improvements included the deletion of YPL062W for increase of the cytosolic pool of acetyl-CA and introduction of the evolutionary mutant of CrtE03M with improved activity. These combined modifications resulted in doubling production of tocotrienol to 5.20 mg/g [51].

It was shown in the study by Ma et al. [54] that expression of the mutant version (attenuated) of geranylgeranyl pyrophosphate synthase GGPPsa of *Sulfolobus acidocaldarius* effectively eliminated inhibition of lycopene cyclase by the substrate, which, together with additional protein engineering of lycopene cyclase itself,facilitated optimal transformation of precursors into β-carotene. Attenuation of the flow mediated by GGPP, helped to balance metabolic flow for lycopene synthesis and its further conversion into β-carotene. At the end, a strain was designed, which was capable of producing 39.5 g/l of β-carotene, which is 1441-fold higher than the initial strain [56].

Yanping Lu et al. [86] used the strategy of stimulation of the entire MVA pathway. It was found, as a result, that overexpression of all genes of the MVA pathway *(ERG10*, *ERG13*, *ERG12*, *ERG8*, *ERG19*, *ERG20*, *tHMG*, and *IDI*) increased production of β-carotene by 46%.

Increase of availability of coenzymes. Heterologous expression of enzymes from other organisms could be ineffective due to the absence of suitable coenzymes and a few intermediates. In particular, cellular metabolism in eukaryotic organisms is divided by the compartments with membranes impermeable to various cofactors and some metabolites. Deficiency of coenzymes in the case of heterologous plant enzymes in yeasts could reduce their activity, and restoration of the synthesis of the required molecules increases efficiency of enzyme operation.

It was shown in the previous studies that the plant enzyme SyHTP exhibits low activity in yeasts. The main reason is the deficit of the NADPH coenzyme [52].

Considering that synthesis of one molecule of IPP or DMAPP via the MVA pathway requires two molecules of NADPH and three molecules of ATP, the genes of the key enzymes of the oxidative branch of pentose phosphate pathway (PPP) and mitochondrial phosphorylation of NADH such as ZWF1 (glucose-6-phosphate dehydrogenase), GND1 (phosphogluconate dehydrogenase), POS5 were overexpressed. Overexpression of these genes increases the intracellular pool of NADPH [23]. It is known that the pentose phosphate pathway is the main source of NADPH formation in cytoplasm. Due to overexpression of the transcription factor Stb5 involved in NADP production it became possible to increase the pool of NADPH and increase production of lycopene to 41.8 mg/g of DCW, which is approximately 1.5-fold higher than in the control stain JHY874D7 (27.2 mg/g of DCW) and 74.6-fold higher than in the initial strain JHY87 (0.56 mg/g of DCW) [87].

Overexpression of the PPP enzymes and regeneration of NADPH – ZWF1, GND1, POS5 – and integration of non-native isopentanol utilization pathway (IUP) increases significantly production of IPP/DMAPP, thus ensuring production of the high titer of lycopene (4.2 g/l) [23].

S-adenosyl-L-methionine (SAM) is the key coenzyme participating in numerous reactions and physiological processes mainly as a donor of methyl group [88]. Furthermore, numerous processes of biosynthesis of natural products include the stage of methylation catalyzed by SAM-dependent methyltransferases [89]. It was shown in many studies that the level of SAM affects the reactions of methylation more than expression of methyltransferases [90].

Introduction of heterologous enzymes, protein engineering. Expression of the genes of heterologous enzymes usually starts with optimization of codons, removal of signal sequences (in the case of plant proteins localized in chloroplasts), combining enzymes from different organisms, which could increase production in comparison with introduction of the enzyme from a single source is also used, as well as fusing proteins for formation of complexes [91], which facilitate targeted use of substrates by decreasing intermediate diffusion and prevent formation of unstable intermediate products.

Codon optimization and truncation of the N-termini of the plant enzymes (MPBQMT, TC, γ-TMT) played a crucial role in improving their functional expression in the yeast cytosol. The complex approach combining truncated enzymes with strategic overexpression (two copies of each gene) resulted in the 8-fold increase of the total production of tocopherols reaching 2.6 mg/g of DCW [51, 92].

Luyao Han and colleagues demonstrated that the design of 16 truncated mutants of TC (tTC) using *Rosetta Cartesian_ddg* resulted in creation of the most effective mutant TCN331P, which increased the δ-tocotrienol titer by 83% [26].

Astaxanthin biosynthesis proceeds through transformation of β-carotene in the four-step reaction with the help of enzymes β-carotene hydroxylase (CrtZ) and β-carotene ketolase (CrtW). Ruizhao Wang with colleagues conducted screening of nine CrtZ enzymes and eight CrtW enzymes from different organisms using *S. cerevisiae* and identified the combination of CrtW from *Brevundimonas vesicularis* DC263 and CrtZ from *Alcaligenes* sp. of the strain PC-1 as the most effective that allowed obtaining the higher astaxanthin titer (81 mg/l) during cultivation in a 5-l fermenter [93].

Han et al. designed protein complexes by combining proteins HPPD, HGGT, and tTC. Screening of the obtained complexes showed that fusion of two of them through a flexible linker (HGGT-GGGGS3-tTC) was more effective than other fused pairs and even fusion of all three enzymes in production of δ-tocotrienol (by 38%) providing yield of 409 mg/l [26]. To further improve yield of tocotrienol, synthetic modules of the HPPD enzyme and the fused complex were developed using SH3, PDZ, and GBD linkers to organize targeted transfer of the substrate; among those the SH3 ligand demonstrated the highest efficiency increasing δ-tocotrienol titer by 156%.

Directing biosynthesis pathways to different compartments could ensure suitable physicochemical conditions and sufficient amount of precursors and enzymes for the complex biosynthesis pathway. It was shown that in the case of tocotrienol synthesis in *S. cerevisiae* the heterologous HGGT from *Synechocystis* sp. PCC 6803 is anchored at the endoplasmic reticulum membrane. In turn, anchoring of the next enzyme in this biosynthesis pathway, cyclase tTC (VTE1), with the help of SEC12 facilitated increase of the δ-tocotrienol titer to 336.7 μg/l. It was suggested that exactly the decrease of distance between the enzymes improved their functioning [26].

With the goal of improving transformation of β-carotene into astaxanthin and minimization of accumulation of intermediate products – zeaxanthin and canthaxanthin – two key enzymes were combined into one polypeptide (PsCrtW-HpCrtZ). This fused gene was supplemented with two signaling sequences targeting the protein to peroxisomes (SKL), lipid bodies (oleosin), and endoplasmic reticulum (KDEL). Cultivation of the obtained strain YL17 for 12 days in flasks under fed-batch conditions resulted in production of astaxanthin with titer 858 mg/l (16.7 mg/g of dry biomass), however, scaling-up of this process to the 3-l bioreactor resulted in reduction of production to 453 mg/l (8.9 mg/g of dry biomass) [67].

The structure-guided protein engineering provided the possibility to eliminate substrate inhibition of the bifunctional phytoene synthase/lycopene cyclase CarRP from *M. circinelloides* during optimization of β-carotene synthesis. Screening of the obtained mutants allowed to identify the CarRP Y27R mutant enzyme, which demonstrated complete absence of inhibition by lycopene without decrease in the enzyme activity and, together with other modifications, allowed producing β-carotene with final titer of 39.5 g/l and 98% selectivity (in comparison with 18% in the wild type) [56].

Zhu and co-authors performed modular assembly of the enzymes using short peptides (RIAD and RIDD) in order to form an enzyme complex of CrtW with CrtZ. Binding stoichiometry of 2 : 1 between RIDD and RIAD provided better results in the case of RIDD addition to CrtW, which allows suggesting that CrtW is a potential rate-limiting factor. The final strain with 20 copies of two genes integrated into the *Y. lipolytica* genome was able to produce the highest described so far yield of astaxanthin of 3.3 g/l during fermentation in a 5-l bioreactor [68].

IDI, last enzyme in the MVA pathway, is localized in cytoplasm, and geranylgeranyl pyrophosphate synthase (CrtE), the first enzyme in carotenoid synthesis pathway, that catalyzes synthesis of the carotenoid precursor GGPP, is associated with the membrane. Also, with the help of the RIDD-RIAD peptide pair the proteins IDI and CrtE were assembled into a single complex in *S. cerevisiae* in order to target IPP/DMAPP directly to the GGPP synthesis thus increasing production of lycopene in *S. cerevisiae* by 58% [76].

Two-step fermentation and extraction of products. Cells in traditional fermentation systems are subjected to metabolic overload, when the cells are simultaneously growing and producing large quantities of secondary metabolites. To obtain high production levels, it is necessary to address the conflict between accumulation of the target product and cell growth [94].

Shen et al. [51] used an innovative system for temperature control induced by the cold shock via modification of the expression of transcription factor Gal4 with the help of its thermo-sensitive variant Gal4M9. The system allowed to separate the phases of growth and production, to ensure high

cell density during fermentation and final tocotrienol titer of 320 mg/l. The system of cold shock uses a complex two-level mechanism of regulation with the thermo-sensitive Gal4M9 controlling expression of the wild type Gal4. In the growth phase at 30°C, the genes of metabolic pathways are practically inactive, which ensures optimal biomass accumulation. Short-term cold shock (24°C for 5 h) activates Gal4M9, which initiates expression of the wild type Gal4. After temperature returning to 30°C, the accumulated Gal4 supports high expression of the genes of metabolic pathways during the entire production phase.

The system of two-phase fermentation was used for effective production of canthaxanthin in the study by Chen et al. [27]. Thermo-sensitive regulatory mechanism of GAL also allowed to separate the stages of biomass accumulation and production of canthaxanthin. In the first stage, the cells were cultivated at 30°C with glucose supplementation. After that, temperature was reduced to 24°C to induce synthesis of canthaxanthin, and carbon source was replaced with ethanol. This strategy ensured production of canthaxanthin with a titer of 1.436 g/l over the period of 260 h in a 5-l bioreactor.

The system of two-phase fermentation in the production astaxanthin in *S. cerevisiae* allowed to reach the product titer of 446.4 mg/l [66].

Jiao et al. [52] used fed-batch fermentation with different carbon sources. This system included the initial stage using a rich glucose-based medium to support rapid growth of the cells. In the second stage of feeding, when the yeast reached the late logarithmic phase, the feeding was switched to ethanol-based medium to support biosynthesis of tocotrienols. Ethanol concentration was maintained at the level lower than the inhibition level (~5 g/l). Dissolved oxygen and pH were thoroughly controlled (in particular, pH 5.5, dissolved oxygen ~40%) with the help of stirring and aeration optimized for the yeast metabolism.

A new approach to fermentation was demonstrated in the recent study – two-step bioconversion of greenhouse gas methane into canthaxanthin using molasses (byproduct of sugar industry) as a carbon source [64]. For this purpose, the *Y. lipolytica* strain producing β-carotene was modified via expression of more than ten genes to introduce pathways of canthaxanthin synthesis, increase of acetyl-CoA flow, and enhance hexose catabolism. A fermentation medium was developed based on biomass of methanotrophic bacterium *Methylococcus capsulatus* (Bath). Scaling-up of the process in a 3-l bioreactor allowed to produce canthaxanthin with titer 1.2 g/l using molasses.

Tocotrienols and carotenoids are hydrophobic compounds, and their excessive accumulation could disrupt cellular homeostasis. To mitigate toxic effect, the possibility of extracellular export of tocotrienols was considered. To achieve maximum efficiency, it is important to select a suitable extractant. Dodecane has been used widely in the two-phase extractive fermentation of various terpenoids [95], and olive oil has been used in the two-phase extractive fermentation of modified yeast for production of vitamin A [96]. Several types of extractants for secretion of tocotrienols into organic phase were tested by Jiao et al. [52] including dodecane and olive oil. The best results were obtained with olive oil, addition of 5% (vol./vol.) of olive oil after 24 h of cultivation resulted in production of a mixture of tocotrienols with yield 76.6 mg/l, in the process 56.12% of the product was secreted into organic phase [52]. In another study, the addition of 50% of dodecane resulted in the maximum titer of the produced δ-tocotrienol (up to 3262.2 μg/l), which was 1.7-fold higher than the product titer produced during the single-phase fermentation [26].

Another approach involves enhancement of lipid accumulation as a cellular reservoir for fat-soluble antioxidants. For example, Ma et al. [54] focused in their study on optimization of triacylglycerols (TAG) in *S. cerevisiae*. Increase of triacylglycerol content was achieved through overexpression of the endogenous enzymes PAH1, DGA1, and the mutant variant of ACC1 encoding ACC1 (S695A, S1175A) under control of strong constitutive promoters P_{PGK1} and P_{TEF1}. This strategy allowed to increase lycopene content by 25%, reaching the record levels of 2.37 g/l during fed-batch fermentation of *S. cerevisiae*; in the process, lycopene was found in cell in the form of droplets and not dispersed in the cytoplasm. Expression of the *HMGR*, *GGS1*, *CarB*, and *CarRP* genes in the lipid-producing strain *Y. lipolytica* resulted in production of β-carotene in the amount of 6.5 g/l (90 mg/g of dry weight) with simultaneous synthesis of 42.6 g/l of lipids [59].

Expression of endogenous transporters. Increase of expression of the corresponding transporters could enhance transmembrane transport of the target product across the cell membrane, thus reducing the cellular load and stimulating its production [97, 98]. It is known that Pdr11 facilitates transport of heterogenous lipophilic products in *S. cerevisiae* [52]. Expression of *Pdr11* in *S. cerevisiae* mediated production of δ-tocotrienol titer of 1965.5 μg/l with extracellular secretion [26].

Secretion of tocotrienols in two-phase fermentation suggests the potential presence of endogenous transporters specific for tocotrienols. However, at present there is no information on the specific for tocotrienol secretory transporters either in natural producers or in yeasts [52]. Xiao Bu with colleagues

in their study overexpressed several types of endogenous PDR proteins, including transporters of hydrophobic compounds such as sterols and carotenoids (Pdr5p, Pdr10p, Pdr11p, Pdr12p, Snq2p, Yor1p, Yol075cp, and Aus1p) and their transcriptional activators (Pdr1p, Pdr3p). It has been reported previously that the enhanced expression of Pdr10p and Yol075cp separately in *S. cerevisiae* significantly increases production and secretion of β-carotene [99]. Indeed, the stains overexpressing precisely Pdr11p and Yol075cp demonstrated the highest capacity for secretion with 1.34- and 1.36-fold increase of extracellular tocotrienols, respectively. In parallel with the increase of secretion efficiency mediated by transporters, production of trienols also increased by 11.92 and 5.15%, which was explained by the accelerated removal of products from the cells. Co-overexpression of Pdr11p and Yol075cp further increased efficiency of secretion. A major fraction of tocotrienols (73.66%) was found in the organic phase, and total content of tocotrienols reached 82.68 mg/l [35]. These results imply that a moderate expression rather than overexpression of transporters is required for optimal growth and secretion [100].

Morphology engineering. The *Y. lipolytica* yeast is a dimorphic yeast existing either in the form of budding cells with a round shape, or in the filamentous (pseudomycelium) form. Although the mycelial form facilitates resistance to harsh conditions, it is not favorable for industrial processes due to effects of its shape on the medium viscosity and efficiency of mass/oxygen transport.

Yeast transition to pseudomycelium and hyphae has been observed in the *Y. lipolytica* strains producing β-carotene with the increase of production levels. Deletion of the serine/threonine protein kinase (CLA4) in the mitogen-activated protein kinase pathway and of the type C2H2 zinc finger transcription factor (MHY1) in the cAMP-protein kinase A pathway resulted in the complete arrest of hyphae growth, which resulted in the increase of the β-carotene titer by 139% without decrease in the growth rate. The designed strain mediated production of β-carotene at the level 7.6 g/l (159 mg/g DCW) in the process of cell cultivation in a periodic culture with stable maintenance of the yeast shape during the entire fermentation process [101].

CONCLUSION

Successful genetic engineering strategies for production of vitamin E and carotenoids in yeast depend of thorough selection and modification of the heterologous biosynthesis enzymes, dynamic regulation of expression of the genes to balance growth and production, removal of bottlenecks in metabolism through increase of the pool and availability of intermediates and coenzymes, fusion of the enzymes for enhancing catalytic efficiency and targeted substrate transport, use of endogenous transporters for excretion of the target products, as well as development and optimization of the fermentation process. The examples of achievements presented above demonstrate high potential of synthetic biology in the creation of yeast cell factories capable of producing biologically active compounds, including antioxidants, with industrially relevant yields.

Abbreviations

4-HPP	4-hydroxyphenylpyruvate
Aro10	phenylpyruvate decarboxylase
Aro3	3-deoxy-7-phosphoheptulonate synthase
bFS	β-farnesene synthase
carB	phytoene dehydrogenase
carRP	phytoene synthase/lycopene β-cyclase
CrtE	geranylgeranyl pyrophosphate synthase
CrtE03M	geranylgeranyl diphosphate synthase
CrtW	β-carotene ketolase
CrtZ	β-carotene hydroxylase
DMAPP	dimethylallyl diphosphate
ERG20	gene of FPP synthase
FPP	farnesyl pyrophosphate
GGPP	geranylgeranyl pyrophosphate
GGPPs	gene of heterologous geranylgeranyl pyrophosphate synthase
GGPPsa	geranylgeranyl pyrophosphate synthase
GGS1	geranylgeranyl pyrophosphate synthase
GPP	geranyl pyrophosphate
HGA	homogentisic acid
HGGT	homogentisate geranylgeranyl transferase
HMG1	3-hydroxy-3-methylglutaryl-CoA reductase
HPPD	4-hydroxyphenylpyruvate dioxygenase
HPT	homogentisate phytyl transferase
HpBKT	β-carotene ketolase of *Haematococcus pluvialis*
HpCrtZ	β-carotene ketolase
IDI	isopentenyl diphosphate isomerase
IPP	isopentenyl diphosphate
KDEL	endoplasmic reticulum retention signal
MPBQ	methyltransferase
MPBQMT	2-methyl-6-phytylbenzoquinol methyltransferase
MVA	mevalonic acid
OBKTM29	β-carotene ketolase

POS5	NADH kinase
SAM	S-adenosyl-L-methionine
ScSUC2	gene of invertase from *S. cerevisiae*
TC	tocopherol cyclase
Tyr1	cyclohexadienyl dehydrogenase
ZWF1	glucose-6-phosphate dehydrogenase
γ-TMT	γ-tocopherol methyltransferase

Contributions

Yu. S. Panina and E. Yuzbasheva – concept and supervision of the study, writing text of the paper, T. Yuzbashev, S. A. Bruskin, L. G. Maloshenok, and S. O. Avdoshina – discussion of the results of the study, editing text of the paper.

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Ethics approval and consent to participate

This work does not contain any studies involving human and animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Ramanathan, N., Tan, E., Loh, L. J., Soh, B. S., and Yap, W. N. (2018) Tocotrienol is a cardioprotective agent against ageing-associated cardiovascular disease and its associated morbidities, *Nutr. Metab.*, **15**, 6, https://doi.org/10.1186/s12986-018-0244-4.
2. Weber, P., Birringer, M., Blumberg, J. B. Eggersdorfer, M., and Frank, J. (2019) Vitamin E, immune function, and protection against infection, in *Vitamin E in Human Health*, pp. 371-384, https://doi.org/10.1007/978-3-030-05315-4.
3. Tan, S. M. Q., Chiew, Y., Ahmad, B., and Kadir, K. A. (2018) Tocotrienol-rich vitamin E from palm oil (tocovid) and its effects in diabetes and diabetic nephropathy: a pilot phase II clinical trial, *Nutrient*, **10**, 1315, https://doi.org/10.3390/nu10091315.
4. Wong, W. Y., Ward, L. C., Fong, C. W., Yap, W. N., and Brown, L. (2017) Anti-inflammatory γ- and δ-tocotrienols improve cardiovascular, liver and metabolic function in diet-induced obese rats, *Eur. J. Nutr.*, **56**, 133-150, https://doi.org/10.1007/s00394-015-1064-1.
5. Stachowiak, B., and Szulc, P. (2021) Astaxanthin for the food industry, *Molecules*, **26**, 2666, https://doi.org/10.3390/molecules26092666.
6. Pangestuti, R., Suryaningtyas, I. T., Siahaan, E. A., and Kim, S.-K. (2020) Cosmetics and Cosmeceutical Applications of Microalgae Pigments, in *Pigments from Microalgae Handbook*, pp. 611-633, https://doi.org/10.1007/978-3-030-50971-2_25.
7. Gaur, V., and Bera, S. (2023) Microbial canthaxanthin: an orange-red keto carotenoid with potential pharmaceutical applications, *J. Biotechnol. Computat. Biol. Bionanotechnol.*, **104**, 315-328, https://doi.org/10.5114/bta.2023.130733.
8. Sathasivam, R., and Ki, J.-S. (2018) A review of the biological activities of microalgal carotenoids and their potential use in healthcare and cosmetic industries, *Marine Drugs*, **16**, 26, https://doi.org/10.3390/md16010026.
9. Lim, K. C., Yusoff, F., Shariff, M., and Kamarudin, M. S. (2018) Astaxanthin as feed supplement in aquatic animals, *Rev. Aquaculture*, **10**, 738-773, https://doi.org/10.1111/raq.12200.
10. El-Sayed, A.-F. M., and Izquierdo, M. (2021) The importance of vitamin E for farmed fish – a review, *Rev. Aquaculture*, **14**, 688-703, https://doi.org/10.1111/raq.12619.
11. Secrist, D. S., Owens, F. N., and Gill, D. R. (1997) Effects of vitamin E on performance of feedlot cattle: a review, *Prof. Animal Sci.*, **13**, 47-54, https://doi.org/10.15232/S1080-7446(15)31844-1.
12. Breithaupt, D. E. (2007) Modern application of xanthophylls in animal feeding – a review, *Trends Food Sci. Technol.*, **18**, 501e506, https://doi.org/10.1016/j.tifs.2007.04.009.
13. Xiao, J., Khan, M. Z., Ma, Y., Alugongo, G. M., Ma, J., Chen, T., Khan, A., and Cao, Z. (2021) The antioxidant properties of selenium and vitamin E; their role in periparturient dairy cattle health regulation, *Antioxidants*, **10**, 1555, https://doi.org/10.3390/antiox10101555.
14. Saini, R. K., and Keum, Y. S. (2019) Microbial platforms to produce commercially vital carotenoids at industrial scale: an updated review of critical issues, *J. Industr. Microbiol. Biotechnol.*, **46**, 657-674, https://doi.org/10.1007/s10295-018-2104-7.
15. Radomska, L. B., and Harasym, J. (2018) β-Carotene – properties and production methods, *Food Quality Safety*, **2**, 69-74, https://doi.org/10.1093/fqsafe/fyy004.
16. Panis, G., and Carreon, J. R. (2016) Commercial astaxanthin production derived by green alga *Haematococcus Pluvialis*: a microalgae process model and a techno-economic assessment all through production line, *Algal Res.*, **18**, 175-190, https://doi.org/10.1016/j.algal.2016.06.007.

17. Moretti, V. M., Mentasti, T., Bellagamba, F., Luzzana, U., Caprino, F., Turchini, G., Giani, I., and Valfrè, F. (2006) Determination of astaxanthin stereoisomers and colour attributes in flesh of rainbow trout (*Oncorhynchus mykiss*) as a tool to distinguish the dietary pigmentation source, *Food Additives Contam.*, **23**, 1056-1063, https://doi.org/10.1080/02652030600838399.
18. Higuera-Ciapara, I., Félix-Valenzuela, L., and Goycoolea, F. M. (2006) Astaxanthin: a review of its chemistry and applications, *Crit. Rev. Food Sci. Nutr.*, **46**, 185-196, https://doi.org/10.1080/10408690590957188.
19. Liu, X., Song, M., Gao, Z., Cai, X., Dixon, W., Chen, X., Cao, Y., and Xiao, H. (2016) Stereoisomers of astaxanthin inhibit human colon cancer cell growth by inducing G2/M cell cycle arrest and apoptosis, *J. Agricult. Food Chem.*, **64**, 7750-7759, https://doi.org/10.1021/acs.jafc.6b03636.
20. Capelli, B., Bagchi, D., and Cysewski, G. R. (2013) Synthetic astaxanthin is significantly inferior to algal-based astaxanthin as an antioxidant and may not be suitable as a human nutraceutical supplement, *Nutrafoods*, **12**, 145-152, https://doi.org/10.1007/s13749-013-0051-5.
21. Ye, Z., Shi, B., Huang, Y., Ma, T., Xiang, Z., Hu, B., Kuang, Z., Huang, M., Lin, X., Tian, Z., Deng, Z., Shen, K., and Liu, T. (2022) Revolution of vitamin E production by starting from microbial fermented farnesene to isophytol, *Innovation*, **3**, 100228, https://doi.org/10.1016/j.xinn.2022.100228.
22. Netscher, T. (2007) Synthesis of vitamin E, *Vitam. Horm.*, **76**, 155-202, https://doi.org/10.1016/S0083-6729(07)76007-7.
23. Jing, Y., Guo, F., Zhang, S., Dong, W., Zhou, J., Xin, F., Zhang, W., and Min, J. (2021) Recent advances on biological synthesis of lycopene by using industrial yeast, *Indust. Eng. Chem. Res.*, **60**, 3485-3494, https://doi.org/10.1021/acs.iecr.0c05228.
24. Kildegaard, K. R., Adiego-Pérez, B., Doménech Belda, D., Khangura, J. K., Holkenbrink, C., and Borodina, I. (2017) Engineering of *Yarrowia Lipolytica* for production of astaxanthin, *Synth. Systems Biotechnol.*, **2**, 287-294, https://doi.org/10.1016/j.synbio.2017.10.002.
25. Xie, W., Lv, X., Ye, L., Zhou, P., and Yu, H. (2015) Construction of lycopene-overproducing *Saccharomyces cerevisiae* by combining directed evolution and metabolic engineering, *Metab. Eng.*, **30**, 69-78, https://doi.org/10.1016/j.ymben.2015.04.009.
26. Han, L., Wu, Y., Xu, Y., Zhang, C., Liu, Y., Li, J., Du, G., Lv, X., and Liu, L. (2024) Engineered *Saccharomyces cerevisiae* for *de novo* δ-tocotrienol biosynthesis, *Systems Microbiol. Biomanufact.*, **4**, 150-164, https://doi.org/10.1007/s43393-023-00167-2.
27. Chen, M., Li, M., Ye, L., and Yu, H. (2022) Construction of canthaxanthin-producing yeast by combining spatiotemporal regulation and pleiotropic drug resistance engineering, *ACS Synth. Biol.*, **11**, 325-333, https://doi.org/10.1021/acssynbio.1c00437.
28. Arora, N., and Pritam, P. (2025) Unlocking yeast's palette: leveraging metabolomics and gene editing approaches for cost effective carotenoid production, *Blue Biotechnol.*, **2**, 5, https://doi.org/10.1186/s44315-025-00028-8.
29. Bureau, J.-A., Oliva, M. E., Dong, Y., and Ignea, C. (2023) Engineering yeast for the production of plant terpenoids using synthetic biology approaches, *Nat. Product Rep.*, **40**, 1822-1848, https://doi.org/10.1039/d3np00005b.
30. Peh, H. Y., Tan, W. S. D., Liao, W., and Wong, W. S. F. (2016) Vitamin E therapy beyond cancer: tocopherol versus tocotrienol, *Pharmacol. Ther.*, **162**, 152-169, https://doi.org/10.1016/j.pharmthera.2015.12.003.
31. Casati, L., Pagani, F., Limonta, P., Vanetti, C., Stancari, G., and Sibilia, V. (2020) Beneficial effects of δ-tocotrienol against oxidative stress in osteoblastic cells: studies on the mechanisms of action european, *J. Nutrit.*, **59**, 1975-1987, https://doi.org/10.1007/s00394-019-02047-9.
32. Ciarcià, G., Bianchi, S., Tomasello, B., Acquaviva, R., Malfa, G. A., Naletova, I., La Mantia, A., and Di Giacomo, C. (2022) Vitamin E and non-communicable diseases: a review, *Biomedicines*, **10**, 2473, https://doi.org/10.3390/biomedicines10102473.
33. He, X., Wang, D., Yi, Y., Tan, Y., Wu, M., Wang, H., Hu, W., Chen, H., Zhang, Q., and Wu, Y. (2023) δ-Tocotrienol preconditioning improves the capability of bone marrow-derived mesenchymal stem cells in promoting wound healing by inhibiting BACH1-related ferroptosis, *Cell Death Discov.*, **9**, 349, https://doi.org/10.1038/s41420-023-01653-1.
34. Ranasinghe, R., Mathai, M., and Zulli, A. (2022) Revisiting the therapeutic potential of tocotrienol, *Biofactors*, **48**, 813-856, https://doi.org/10.1002/biof.1873.
35. Gamna, F., and Spriano, S. (2021) Vitamin E: a review of its application and methods of detection when combined with implant biomaterials, *Materials (Basel)*, **14**, 3691, https://doi.org/10.3390/ma14133691.
36. Mène-Saffrané, L. (2017) Vitamin E Biosynthesis and its regulation in plants, *Antioxidants*, **7**, 2, https://doi.org/10.3390/antiox7010002.
37. Muñoz, P., and Munné-Bosch, S. (2019) Vitamin E in plants: biosynthesis, transport, and function, *Trends Plant Sci.*, **24**, 1040-1051, https://doi.org/10.1016/j.tplants.2019.08.006.

38. Ashokkumar, V., Flora, G., Sevanan, M., Sripriya, R., Chen, W. H., Park, J.-H., Rajesh Banu, J., and Kumar, G. (2023) Technological advances in the production of carotenoids and their applications – a critical review, *Biores. Technol.*, **367**, 128215, https://doi.org/10.1016/j.biortech.2022.128215.
39. Yabuzaki, J. (2017) Carotenoids database: structures, chemical fingerprints and distribution among organisms, *Database*, **2017**, bax004, https://doi.org/10.1093/database/bax004.
40. Patel, A. K., Tambat, V. S., Chen, C.-W., Chauhan, A. S., Kumar, P., Vadrale, A. P., Huang, C.-Y., Dong, C.-D., and Singhania, R. R. (2022) Recent advancements in astaxanthin production from microalgae: a review, *Biores. Technol.*, **364**, 128030, https://doi.org/10.1016/j.biortech.2022.128030.
41. Wang, Z., Sun, J., Yang, Q., and Yang, J. (2020) Metabolic engineering *Escherichia coli* for the production of lycopene, *Molecules*, **25**, 3136, https://doi.org/10.3390/molecules25143136.
42. Esatbeyoglu, T., and Rimbach, G. (2017) Canthaxanthin: from molecule to function, *Mol. Nutr. Food Res.*, **61**, 1600469, https://doi.org/10.1002/mnfr.201600469.
43. Kuzuyama, T., and Seto, H. (2012) Two distinct pathways for essential metabolic precursors for isoprenoid biosynthesis, *Proc. Japan Acad. Ser. B*, **88**, 41-52, https://doi.org/10.2183/pjab.88.41.
44. Zhou, T., Park, Y.-K., and Ledesma-Amaro, R. (2025) Advances in the biosynthesis of β-carotene and its derivatives in yeast, *Biores. Technol.*, **435**, 132936, https://doi.org/10.1016/j.biortech.2025.132936.
45. Li, C., Swofford, C. A., and Sinskey, A. J. (2020) Modular engineering for microbial production of carotenoids, *Metab. Eng. Commun.*, **10**, e00118, https://doi.org/10.1016/j.mec.2019.e00118.
46. Zhang, X. K., Wang, D. N., Chen, J., Liu, Z. J., Wei, L. J., and Hua, Q. (2020) Metabolic engineering of β-carotene biosynthesis in *Yarrowia lipolytica*, *Biotechnol. Lett.*, **42**, 945-956, https://doi.org/10.1007/s10529-020-02844-x.
47. Velayos, A., Eslava, A. P., and Iturriaga, E. A. (2000) A bifunctional enzyme with lycopene cyclase and phytoene synthase activities is encoded by the carRP gene of mucor circinelloides, *Eur. J. Biochem.*, **267**, 5509-5519, https://doi.org/10.1046/j.1432-1327.2000.01612.x.
48. Tramontin, L. R. R., Kildegaard, K. R., Sudarsan, S., and Borodina, I. (2019) Enhancement of astaxanthin biosynthesis in oleaginous yeast *Yarrowia Lipolytica* via microalgal pathway, *Microorganisms*, 7, 472, https://doi.org/10.3390/microorganisms7100472.
49. Runguphan, W., and Keasling, J. D. (2014) Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid-derived biofuels and chemicals, *Metab. Eng.*, **21**, 103-113, https://doi.org/10.1016/j.ymben.2013.07.003.
50. Sun, H., Yang, J., Lin, X., Li, C., He, Y., Cai, Z., Zhang, G., and Song, H. (2020) *De novo* high-titer production of delta-tocotrienol in recombinant *Saccharomyces cerevisiae*, *J. Agricult. Food Chem.*, **68**, 7710-7717, https://doi.org/10.1021/acs.jafc.0c00294.
51. Shen, B., Zhou, P., Jiao, X., Yao, Z., Ye, L., and Yu, H. (2020) Fermentative production of vitamin E tocotrienols in *Saccharomyces cerevisiae* under cold-shock-triggered temperature control, *Nat. Commun.*, **11**, 5155, https://doi.org/10.1038/s41467-020-18958-9.
52. Jiao, X., Shen, B., Li, M., Ye, L., and Yu, H. (2022) Secretory production of tocotrienols in *Saccharomyces Cerevisiae*, *ACS Synth. Biol.*, **11**, 788-799, https://doi.org/10.1021/acssynbio.1c00484.
53. Xiang, J., Liu, M., Wang, X., Yue, M., Qin, Z., and Zhou, J. (2025) Combined metabolic and enzymatic engineering for de novo biosynthesis of δ-tocotrienol in *Yarrowia lipolytica*, *Synth. Systems Biotechnol.*, **10**, 719-727, https://doi.org/10.1016/j.synbio.2025.02.011.
54. Ma, T., Shi, B., Ye, Z., Li, X., Liu, M., Chen, Y., Xia, J., Nielsen, J., Deng, Z., and Liu, T. (2019) Lipid engineering combined with systematic metabolic engineering of *Saccharomyces cerevisiae* for high-yield production of lycopene, *Metab. Eng.*, **52**, 134-142, https://doi.org/10.1016/j.ymben.2018.11.009.
55. Xu, X., Liu, J., Lu, Y., Lan, H., Tian, L., Zhang, Z., Xie, C., and Jiang, L. (2021) Pathway engineering of *Saccharomyces cerevisiae* for efficient lycopene production, *Bioproc. Biosyst. Eng.*, **44**, 1033-1047, https://doi.org/10.1007/s00449-020-02503-5.
56. Ma, Y., Liu, N., Greisen, P., Li, J., Qiao, K., Huang, S., and Stephanopoulos, G. (2022) Removal of lycopene substrate inhibition enables high carotenoid productivity in *Yarrowia Lipolytica*, *Nat. Commun.*, **13**, 572, https://doi.org/10.1038/s41467-022-28277-w.
57. Fathi, Z., Tramontin, L. R. R., Ebrahimipour, G., Borodina, I., and Darvishi, F. (2021) Metabolic engineering of *Saccharomyces cerevisiae* for production of β-carotene from hydrophobic substrates, *FEMS Yeast Res.*, **21**, foaa068, https://doi.org/10.1093/femsyr/foaa068.
58. Gao, S., Tong, Y., Zhu, L., Ge, M., Zhang, Y., Chen, D., Jiang, Y., and Yang, S. (2017) Iterative integration of multiple-copy pathway genes in *Yarrowia Lipolytica* for heterologous β-carotene production, *Metab. Eng.*, **41**, 192-201, https://doi.org/10.1016/j.ymben.2017.04.004.

59. Larroude, M., Celinska, E., Back, A., Thomas, S., Nicaud, J. M., and Ledesma-Amaro, R. (2018) A synthetic biology approach to transform *Yarrowia lipolytica* into a competitive biotechnological producer of β-carotene, *Biotechnol. Bioeng.*, **11**, 464-472, https://doi.org/10.1002/bit.26473.
60. Taratynova, M. O., Kosikhina, Y. M., Vinogradova, E. B., Dementev, D. A., Korobov, V. S., Zolottsev, V. A., Yuzbashev, T. V., Yuzbasheva, E. Y., and Sineoky, S. P. (2021) Accumulation of neutral lipids and β-carotene by the *Yarrowia lipolyt*ica yeast on a medium with sucrose as a carbon source, *Biotechnology*, **37**, 29-41, https://doi.org/10.21519/0234-2758-2021-37-3-29-41.
61. Taratynova, M. O., Tikhonova, E. E., Fedyaeva, Iu. M., Dementev, D. A., Yuzbashev, T. V., Solovyev, A. I., Sineoky, S. P., and Yuzbasheva, E. Y. (2023) Boosting geranyl diphosphate synthesis for linalool production in engineered *Yarrowia lipolytica*, *Appl. Biochem. Biotechnol.*, **196**, 1304-1315, https://doi.org/10.1007/s12010-023-04581-z.
62. Yuzbashev, T. V., Yuzbasheva, E. Y., Melkina, O. E., Patel, D., Bubnov, D., Dietz, H., and Ledesma-Amaro, R. A. (2023) A DNA assembly toolkit to unlock the CRISPR/Cas9 potential for metabolic engineering, *Commun. Biol.*, **6**, 858, https://doi.org/10.1038/s42003-023-05202-5.
63. Cui, Z., Zheng, H., Zhang, J., Jiang, Z., Zhu, Z., Liu, X., Qi, Q., and Hou, J. (2021) A CRISPR/Cas9-mediated, homology-independent tool developed for targeted genome integration in *Yarrowia lipolytica*, *Appl. Environ. Microbiol.*, **87**, e02666-20, https://doi.org/10.1128/AEM.02666-20.
64. Taratynova, M. O., Tarasov, I. M., Fedyaeva, I. M., Dementev, D. A., Gorchakova, V. A., Tarasova, M. A., Fedorov, A. S., Yuzbashev, T., Sineoky, S. P., and Yuzbasheva, E. Y. (2025) A two-step process for converting methane to canthaxanthin using *Methylococcus capsulatus* (bath) biomass and engineered *Yarrowia lipolytica*, *Biotechnol. J.*, **20**, e70043, https://doi.org/10.1002/biot.70043.
65. Jin, D., Gu, B., Xiong, D., Huang, G., Huang, X., Lan, L., and Xiao, J. (2018) A transcriptomic analysis of *Saccharomyces cerevisiae* under the stress of 2-phenylethanol, *Curr. Microbiol.*, **75**, 1068-1076, https://doi.org/10.1007/s00284-018-1488-y.
66. Li, M., Zhou, P., Chen, M., Yu, H., and Ye, L. (2022) Spatiotemporal regulation of astaxanthin synthesis in *S. cerevisiae*, *ACS Synth. Biol.*, **11**, 2636-2649, https://doi.org/10.1021/acssynbio.2c00044.
67. Ma, Y., Li, J., and Huang, S. (2021) Targeting pathway expression to subcellular organelles improves astaxanthin synthesis in *Yarrowia Lipolytica*, *Metab. Eng.*, **68**, 152-161, https://doi.org/10.1016/j.ymben.2021.10.004.
68. Zhu, H. Z., Jiang, S., Wu, J. J., Zhou, X. R., Liu, P. Y., Huang, F. H., and Wan, X. (2022) Production of high levels of 3S,3'S-astaxanthin in *Yarrowia lipolytica* via iterative metabolic engineering, *J. Agricult. Food Chem.*, **70**, 2673-2683, https://doi.org/10.1021/acs.jafc.1c08072.
69. Abdullah, C. N., Liu, M., Chen, Q., Gao, S., Zhang, C., Liu, S., and Zhou, J. (2025) Efficient production of astaxanthin in *Yarrowia lipolytica* through metabolic and enzyme engineering, *Synth. Syst. Biotechnol.*, **10**, 737-750, https://doi.org/10.1016/j.synbio.2025.02.014.
70. Sertil, O., Kapoor, R., Cohen, B. D., Abramova, N., and Lowry, C. V. (2003) Synergistic repression of anaerobic genes by Mot3 and Rox1 in *Saccharomyces cerevisiae*, *Nucleic Acids Res.*, **31**, 5831-5837, https://doi.org/10.1093/nar/gkg792.
71. Trikka, F. A., Nikolaidis, A., Athanasakoglou, A., Andreadelli, A., Ignea, C., Kotta, K., Argiriou, A., Kampranis, S. C., and Makris, A. M. (2015) Iterative carotenogenic screens identify combinations of yeast gene deletions that enhance sclareol production, *Microb. Cell Factor.*, **14**, 60, https://doi.org/10.1186/s12934-015-0246-0.
72. Geiselman, G. M., Zhuang, X., Kirby, J., Tran-Gyamfi, M. B., Prahl, J. P., Sundstrom, E. R., Gao, Y., Munoz, N., Nicora, C. D., Clay, D. M., Papa, G., Burnum-Johnson, K. E., Magnuson, J. K., Tanjore, D., Skerker, J. M., and Gladden, J. M. (2020) Production of ent-kaurene from lignocellulosic hydrolysate in *Rhodosporidium toruloides*, *Microb. Cell Fact.*, **19**, 24, https://doi.org/10.1186/s12934-020-1293-8.
73. Vidi, P. A., Kanwischer, M., Baginsky, S., Austin, J. R., Csucs, G., Dörmann, P., Kessler, F., and Bréhélin, C. (2006) Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplast plastoglobule lipoprotein particles, *J. Biol. Chem.*, **281**, 11225-11234, https://doi.org/10.1074/jbc.M511939200.
74. Liu, M., Wang, C., Ren, X., Gao, S., Yu, S., and Zhou, J. (2022) Remodelling metabolism for high-level resveratrol production in *Yarrowia lipolytica*, *Biores. Technol.*, **365**, 128178, https://doi.org/10.1016/j.biortech.2022.128178.
75. Sun, L., Atkinson, C. A., Lee, Y. G., and Jin, Y. S. (2020) High-level β-carotene production from xylose by engineered *Saccharomyces cerevisiae* without overexpression of a truncated HMG1 (tHMG1), *Biotechnol. Bioeng.*, **117**, 3522-3532, https://doi.org/10.1002/bit.27508.
76. Kang, W., Ma, T., Liu, M., Qu, J., Liu, Z., Zhang, H., Shi, B., Fu, S., Ma, J., Lai, L. T. F., He, S., Qu, J., Au, S. W.-N., Kang, B. H., Lau, W. C. Y., Deng, Z., Xia, J., and Liu, T. (2019) Modular enzyme assembly for enhanced cascade biocatalysis and metabolic flux, *Nat. Commun.*, **10**, 4248, https://doi.org/10.1038/s41467-019-12247-w.

77. Schnappauf, G., Hartmann, M., Künzler, M., and Braus, G. H. (1998) The two 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase isoenzymes from *Saccharomyces cerevisiae* show different kinetic modes of inhibition, *Arch. Microbiol.*, **169**, 517-524, https://doi.org/10.1007/s002030050605.
78. Hartmann, M., Schneider, T. R., Pfeil, A., Heinrich, G., Lipscomb, W. N., and Braus, G. H. (2003) Evolution of feedback-inhibited β/α barrel isoenzymes by gene duplication and a single mutation, *Proc. Natl. Acad. Sci. USA*, **100**, 862-867, https://doi.org/10.1073/pnas.0337566100.
79. Koopman, F., Beekwilder, J., Crimi, B., van Houwelingen, A., Hall, R. D., Bosch, D., van Maris, A. J., Pronk, J. T., and Daran, J. M. (2012) De novo production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*, *Microb. Cell Fact.*, **11**, 155, https://doi.org/10.1186/1475-2859-11-155.
80. Zhao, G., Xia, T., Ingram, L. O., and Jensen, R. A. (1993) An allosterically insensitive class of cyclohexadienyl dehydrogenase from *Zymomonas mobilis*, *Eur. J. Biochem.*, **212**, 157-165, https://doi.org/10.1111/j.1432-1033.1993.tb17646.x.
81. Liu, Q., Yu, T., Li, X., Chen, Y., Campbell, K., Nielsen, J., and Chen, Y. (2019) Rewiring carbon metabolism in yeast for high level production of aromatic chemicals, *Nat. Commun.*, **10**, 4976, https://doi.org/10.1038/s41467-019-12961-5.
82. Norris, S. R., Shen, X., and DellaPenna, D. (1998) Complementation of the *Arabidopsis* pds1 mutation with the gene encoding p-hydroxyphenylpyruvate dioxygenase, *Plant Physiol.*, **117**, 1317-1323, https://doi.org/10.1104/pp.117.4.1317.
83. Martínez, L. M., Martinez, A., and Gosset, G. (2019) Production of melanins with recombinant microorganisms, *Front. Bioeng. Biotechnol.*, **7**, 285, https://doi.org/10.3389/fbioe.2019.00285.
84. Dai, Z., Liu, Y., Huang, L., and Zhang, X. (2012) Production of miltiradiene by metabolically engineered *Saccharomyces cerevisiae*, *Biotechnol. Bioeng.*, **109**, 2845-2853, https://doi.org/10.1002/bit.24547.
85. Liu, J., Zhang, W., Du, G., Chen, J., and Zhou, J. (2013) Overproduction of geraniol by enhanced precursor supply in *Saccharomyces cerevisiae*, *J. Biotechnol.*, **168**, 446-451, https://doi.org/10.1016/j.jbiotec.2013.10.017.
86. Lu, Y., Yang, Q., Lin, Z., and Yang, X. (2020) A modular pathway engineering strategy for the high-level production of β-ionone in *Yarrowia lipolytica*, *Microb. Cell Fact.*, **19**, 49, https://doi.org/10.1186/s12934-020-01309-0.
87. Hong, J., Park, S.-H., Kim, S., Kim, S.-W., and Hahn, J.-S. (2019) Efficient production of lycopene in *Saccharomyces cerevisiae* by enzyme engineering and increasing membrane flexibility and NAPDH production, *Appl. Microbiol. Biotechnol.*, **103**, 211-223, https://doi.org/10.1007/s00253-018-9449-8.
88. Malakar, D., Dey, A., and Ghosh, A. K. (2006) Protective role of S-adenosyl-l-methionine against hydrochloric acid stress in *Saccharomyces cerevisiae*, *Biochim. Biophys. Acta*, **1760**, 1298-1303, https://doi.org/10.1016/j.bbagen.2006.07.004.
89. Zou, X. W., Liu, Y. C., Hsu, N. S., Huang, C. J., Lyu, S. Y., Chan, H. C., Chang, C. Y., Yeh, H. W., Lin, K. H., Wu, C. J., Tsai, M. D., and Li, T. L. (2014) Structure and mechanism of a nonhaem-iron SAM-dependent C-methyltransferase and its engineering to a hydratase and an O-methyltransferase, *Acta Crystallogr. D Struct. Biol.*, **70**, 1549-1560, https://doi.org/10.1107/S1399004714005239.
90. Kunjapur, A. M., Hyun, J. C., and Prather, K. L. (2016) Deregulation of S-adenosylmethionine biosynthesis and regeneration improves methylation in the *E. coli de novo* vanillin biosynthesis pathway, *Microb. Cell Fact.*, **15**, 61, https://doi.org/10.1186/s12934-016-0459-x.
91. Chen, R., Yang, S., Zhang, L., and Zhou, Y. J. (2020) Advanced strategies for production of natural products in yeast, *iScience*, **23**, 100879, https://doi.org/10.1016/j.isci.2020.100879.
92. Peng, B., Bandari, N. C., Lu, Z., Howard, C. B., Scott, C., Trau, M., Dumsday, G., and Vickers, C. E. (2022) Engineering eukaryote-like regulatory circuits to expand artificial control mechanisms for metabolic engineering in *Saccharomyces cerevisiae*, *Commun. Biol.*, **5**, 135, https://doi.org/10.1038/s42003-022-03070-z.
93. Wang, R., Gu, X., Yao, M., Pan, C., Liu, H., Xiao, W., Wang, Y., and Yuan, Y. (2017) Engineering of β-carotene hydroxylase and ketolase for astaxanthin overproduction in *Saccharomyces cerevisiae*, *Front. Chem. Sci. Eng.*, **11**, 89-99, https://doi.org/10.1007/s11705-017-1628-0.
94. Wu, G., Yan, Q., Jones, J. A., Tang, Y. J., Fong, S. S., and Koffas, M. A. G. (2016) Metabolic burden: cornerstones in synthetic biology and metabolic engineering applications, *Trends Biotechnol.*, **34**, 652-664, https://doi.org/10.1016/j.tibtech.2016.02.010.
95. Jang, H. J., Yoon, S. H., Ryu, H. K., Kim, J. H., Wang, C. L., Kim, J. Y., Oh, D. K., and Kim, S. W. (2011) Retinoid production using metabolically engineered Escherichia coli with a two-phase culture system, *Microb. Cell Fact.*, **10**, 59, https://doi.org/10.1186/1475-2859-10-59.
96. Anter, J., Campos-Sánchez, J., Hamss, R. E., Rojas-Molina, M., Muñoz-Serrano, A., Analla, M., and Alonso-Moraga, A. (2010) Modulation of genotoxicity by Extra-virgin olive oil and some of its distinctive components assessed

by use of the Drosophila wing-spot test, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, **703**, 137-142, https://doi.org/10.1016/j.mrgentox.2010.08.012.
97. Lee, J. J., Chen, L., Cao, B., and Chen, W. N. (2016) Engineering *Rhodosporidium toruloides* with a membrane transporter facilitates production and separation of carotenoids and lipids in a bi-phasic culture, *Appl. Microbiol. Biotechnol.*, **100**, 869-877, https://doi.org/10.1007/s00253-015-7102-3.
98. Dunlop, M. J., Dossani, Z. Y., Szmidt, H. L., Chu, H. C., Lee, T. S., Keasling, J. D., Hadi, M. Z., and Mukhopadhyay, A. (2011) Engineering microbial biofuel tolerance and export using efflux pumps, *Mol. Syst. Biol.*, 7, 487, https://doi.org/10.1038/msb.2011.21.
99. Bu, X., Lin, J. Y., Cheng, J., Yang, D., Duan, C. Q., Koffas, M., and Yan, G. L. (2020) Engineering endogenous ABC transporter with improving ATP supply and membrane flexibility enhances the secretion of β-carotene in *Saccharomyces cerevisiae*, *Biotechnol. Biofuels*, **13**, 168, https://doi.org/10.1186/s13068-020-01809-6.
100. Turner, W. J., and Dunlop, M. J. (2015) Trade-offs in improving biofuel tolerance using combinations of efflux pumps, *ACS Synth. Biol.*, **4**, 1056-1063, https://doi.org/10.1021/sb500307w.
101. Liu, M., Zhang, J., Ye, J., Qi, Q., and Hou, J. (2021) Morphological and metabolic engineering of *Yarrowia lipolytica* to increase β-carotene production, *ACS Synth. Biol.*, **10**, 3551-3560, https://doi.org/10.1021/acssynbio.1c00480.