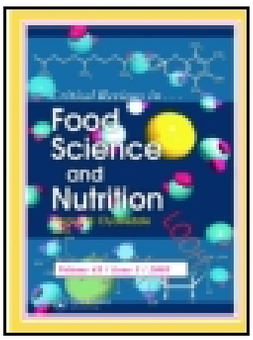

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REVIEW

Yarrowia lipolytica as an emerging biotechnological chassis for functional sugars biosynthesis

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ABSTRACT

Functional sugars have unique structural and physiological characteristics with applied perspectives for modern biomedical and biotechnological sectors, such as biomedicine, pharmaceutical, cosmeceuticals, green chemistry, and agro-food. They can also be used as starting matrices to produce biologically active metabolites of interests. Though numerous chemical synthesis routes have been proposed and deployed for the synthesis of rare sugars, however, many of them are limited and economically incompetent because of expensive raw starting feedstocks. Whereas, the biosynthesis by enzymatic means are often associated with high catalyst costs and low space-time yields. Microbial production of rare sugars via green routes using bio-renewable resources offers noteworthy solutions to overcome the aforementioned limitations of synthetic and enzymatic synthesis routes. From the microbial-based synthesis perspective, the lipogenic yeast *Yarrowia lipolytica* is rapidly evolving as the most prevalent and unique "non-model organism" in the bio-production arena. Due to high flux tendency through the tri-carboxylic acid cycle intermediates and precursors such as acetyl-CoA and malonyl-CoA, this yeast has been widely investigated to meet the increasing demand of industrially relevant fine chemicals, including functional sugars. Incredible interest in *Y. lipolytica* originates from its robust tolerance to unstable pH, salt levels, and organic compounds, which subsequently enable easy bioprocess optimization. Meaningfully, GRAS (generally recognized as safe) status creates *Y. lipolytica* as an attractive and environmentally friendly microbial host for the manufacturing of nutraceuticals, fermented food, and dietary supplements. In this review, we highlight the recent and state-of-the-art research progress on *Y. lipolytica* as a host to synthesize bio-based compounds of interest beyond the realm of well-known fatty acid production. The unique physicochemical properties, biotechnological applications, and biosynthesis of an array of value-added functional sugars including erythritol, threitol, fructooligosaccharides, galactooligosaccharides, isomalto-oligosaccharides, isomaltulose, trehalose, erythrulose, xylitol, and mannitol using sustainable carbon sources are thoroughly vetted. Finally, we conclude with perspectives that would be helpful to engineer *Y. lipolytica* in greening the twenty-first century biomedical and biotechnological sectors of the modern world.

KEYWORDS

Biocatalysis; biotransformation; functional sugars; industrial biotechnology; metabolic engineering; *Yarrowia lipolytica*

Yarrowia lipolytica – an emerging host with unique production potential

Production of industrially pertinent chemicals and biomaterials that keep modern society afloat predominantly depends on the conversion of fossil fuel-derived carbon. A limited supply of fossil fuel precursors and rising environmental concerns associated with the use of petrochemicals are the strategic drivers to develop sustainable and renewable production methods. In this avenue, microbial fermentation using bio-renewable substrates has been recognized as an emerging way to circumvent these traditional chemical synthesis methods. Particularly, bio-manufacturing processes operate under mild reaction environments, utilizes renewable resources, and promote high enantiomeric selectivity through biosynthetic reactions. Extensive work, over the last many years, has augmented the utilization of second-generation carbon sources such as nonfood crops and agricultural waste streams that facilitate an immense sustainability level for the production of bio-based chemicals (Aditiya et al. 2016; Ekas, Deaner, and Alper 2019).

An array of conventional organisms has been used for the biosynthesis of a diversity of different renewable biochemical (Becker, Rohles, and Wittmann 2018; Gu et al. 2018; Pontrelli et al. 2018). Among these organisms, some non-conventional hosts, particularly, *Yarrowia lipolytica* have received popularity, among academia, researches and industrialists, as a biotechnological workhorse in diverse applications. Incredible interest in oleaginous yeast *Y. lipolytica* stems from its robust tolerance to fluctuating pH, salt

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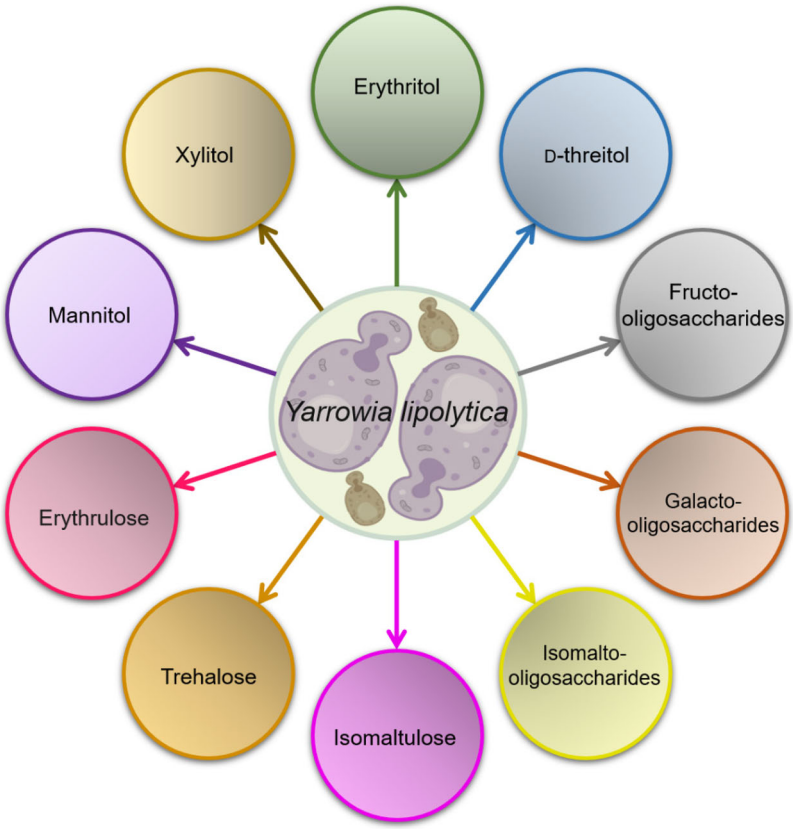

Figure 1. An array of value-added functional sugars obtained from *Yarrowia lipolytica*.

concentrations, and a wide range of organic compounds, which subsequently simplify bioprocess optimization and facilitates the utilization of non-glucose based feedstock's (Miller and Alper 2019). Additionally, a large set of tools for genetic manipulations enables rational engineering of this host and thus directing the elevated flux through the pentose phosphate pathway (PPP) and high acetyl-CoA pools. More importantly, GRAS (generally recognized as safe) status constitutes *Y. lipolytica* as an attractive and environmentally friendly microbial host for the manufacturing of nutraceuticals, fermented food and dietary supplements (Markham and Alper 2018).

As a typical oleaginous yeast, *Y. lipolytica* has been widely investigated for the production of many valuable compounds such as fatty acid-based chemicals, carotenoids, fuels, natural metabolites, enzymes, polyhydroxyalkanoates, various proteins, methyl ketones, mannitol, erythritol, and organic acids (Blazeck et al. 2015; Celińska et al. 2018; Dulermo et al. 2017; Fickers, Marty, and Nicaud 2011; Hanko et al. 2018; Kubiak et al. 2019; Ledesma-Amaro and Nicaud 2016; Vandermies and Fickers 2019; Xie 2017; Yan et al. 2018). This yeast has also been tailored to synthesize fragrance molecules such as γ-decalactone and 2-phenyl ethanol (Braga and Belo 2016; Celińska et al. 2013). In this review, we highlight the recent and state-of-the-art progress in the use of *Y. lipolytica* to synthesize an array of value-added functional sugars (Figure 1) along with their physicochemical properties and biotechnological applications.

Erythritol – functional features and applications

Erythritol is a simple four-carbon linear polyol, each carrying a one-hydroxyl group. It is a natural sweetener (75% as sweet as sucrose) and commonly occurs as a storage compound or metabolite in many fermented foods, fruits, wine, honey, mushrooms, and seaweeds (Goossens and Roeper 1994; Moon et al. 2010). Erythritol has a sweet taste without leveraging aftertaste bitterness and possesses a relatively lower dietary energy content than sucrose. Therefore, it may be consumed in combination with other strong artificial sweeteners, such as aspartame, that linger a bitter aftertaste (Tomaszewska, Rywińska, and Gładkowski 2012). Due to these impressive properties, erythritol finds broad-spectrum applications as an ingredient in beverages, foods, and pharmaceuticals (Haas, Haas, and Tiefenbacher 2010; Lee et al. 2012). In addition, it can also be employed as an intermediate in synthesizing mannosylerythritol lipid that is an antiaging ingredient with extensive consumption in quasi-drugs and cosmetics (Michiko et al. 2009).

Chemically, di-aldehyde starch can be used to produce erythritol in a high-temperature reaction using metal as a catalyst; however, this process implicates many sequential steps rendering it very expensive to be industrialized (Lee et al. 2010). For these reasons, currently, erythritol is industrially synthesized by microbial fermentative processes using various bacterial and yeast strains such as *Aureobasidium*, *C. magnoliae*, *Pseudozyma tsukubaensis*, *Moniliella sp.*,

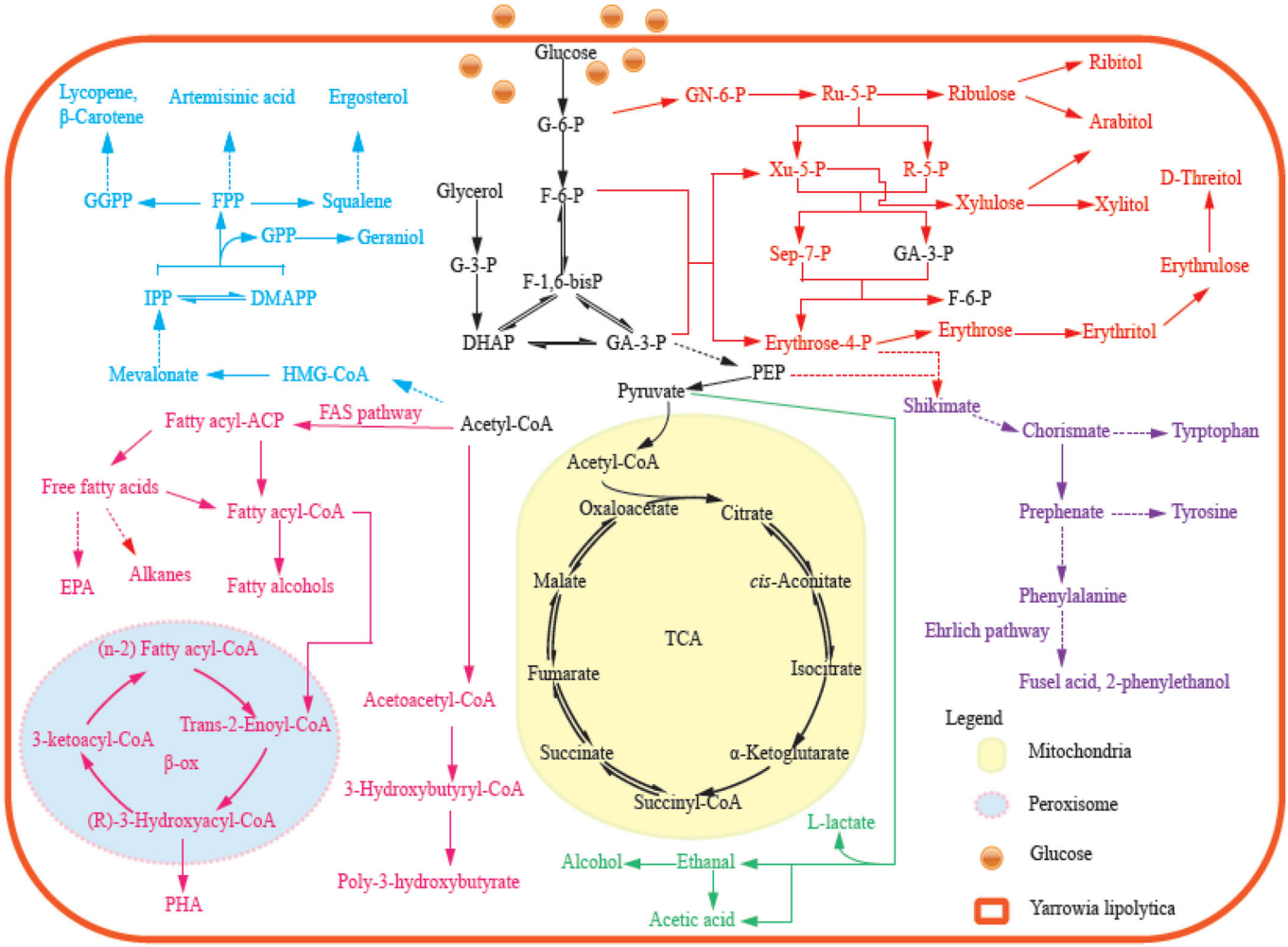

Figure 2. Schematic representation of the primary metabolic pathway for erythritol production in *Yarrowia lipolytica*.
Abbreviations: TCA: tricarboxylic acid cycle; G-3-P: glycerol-3-phosphate; DHAP: dihydroxyacetone phosphate; GA-3-P: glyceraldehyde-3-phosphate; fructose-1,6-bisP: fructose-1,6-bisphosphate; fructose-6-P: fructose-6-phosphate; G-6-P: glucose-6- phosphate; PEP: phosphoenolpyruvic acid; GN-6-P: gluconolactone-6-phosphate; Ru-5-P: ribulose-5- phosphate; Xu-5-P: xylulose-5- phosphate; R-5-P: ribose-5- phosphate; Sep-7-P: sedoheptulose-7- phosphate; erythrose-4-P: erythrose-4-phosphate; GGPP: geranylgeranyl diphosphate; FPP: farnesyl diphosphate; GPP: geranyl diphosphate; IPP: isopentenyl pyrophosphate; DMAPP: dimethylallyl pyrophosphate; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; FAS: fatty acid synthesis; EPA: eicosapentaenoic acid; PHA: polyhydroxyalkanoates; *β*-ox: beta-oxidation.

Trichosporonoides megachiliensis, *Torula corallina*, and *Y. lipolytica* (Ishizuka et al. 1989; Janek et al. 2017; Jeya et al. 2009; Koh et al. 2003; Sawada et al. 2009; Cheng et al. 2018). Due to the GRAS status, *Y. lipolytica* is regarded as an efficient erythritol producer and is capable of utilizing various carbon sources i.e. alkanes, crude glycerol, or fatty acids (Zhu and Jackson 2015). Though the crude form of glycerol comprises a variety of undesired contaminations such as salts, methanol, or heavy metals, this oleaginous yeast can easily assimilate this substrate in spite of the presence of contaminants (Dobrowolski et al. 2016).

Recently, Mironczuk, Biegalska, and Dobrowolski (2017) revealed the major metabolic pathways for erythritol production using glycerol and glucose as a substrate in yeast *Y. lipolytica*. They documented that erythritol production occurs via the PPP, where the transketolase enzyme plays the most important role since the gene overexpression encoding this enzyme resulted in an enhancement (2-fold) in erythritol titer during shake-flasks based experiment. Additionally, the last step of the process requires a reducing agent, which is supplied by glucose-6-phosphate dehydrogenase. It is shown that the addition of glycerol markedly increased the production of erythritol in the media containing crude glycerol and yeast extract (Tomaszewska, Rywińska, and Rymowicz 2014a; Tomaszewska, Rymowicz, and Rywińska 2014b). Glycerol in *Y. lipolytica* cells is consumed via a phosphorylation pathway, where it is phosphorylated to 3-P-glycerol by a glycerol kinase (GK) followed by dehydrogenated to DHAP (Makri, Fakas, and Aggelis 2010). Afterward, the dephosphorylation of erythrose-4-phosphate gives rise to erythrose via the PPP. The resultant erythrose is finally reduced to erythritol by a step catalyzed by ER with the concomitant oxidation of NAD(P)H (Moon et al. 2010). In silico analysis revealed many ER homologous in the genome of *Y. lipolytica* belonging to a large superfamily of Aldo-ketoreductases with more than 40 different members (Ellis 2002). To get a deeper insight into the erythritol biosynthesis pathway, Mirończuk et al. (2015) sequenced the *Y. lipolytica* genomes and demonstrated the occurrence of numerous homologs proteins

Table 1. Comparison of erythritol concentrations, yields and productivities in a different mode of fermentation by various *Yarrowia lipolytica* strains.

Microorganism	Strategy	Mode of process	Erythritol (g L^{-1})	Yield (g g^{-1})	Productivity (g L^{-1} h^{-1})	References
Y. lipolytica	Overexpression of codon-optimized bacterial hemoglobin from *Vitreoscilla stercoraria*	Bioreactor culture	55.75	0.37	0.38	Mirończuk et al. (2019)
Y. lipolytica	Overexpression of GUT1 and TKL1 Disruption of EYK1	Bioreactor cultures	80.6	0.53	1.03	Carly et al. (2017a, 2017b)
Y. lipolytica MK1	Ultraviolet mutagenesis and optimal C:N ratio	Chemostat culture	113.1	0.57	1.14	Rakicka et al. (2017)
Y. lipolytica	Overexpression of gene *YALI0F18590*g encoding the erythrose reductase	Batch culture	44.44	0.44	0.77	Janek et al. (2017)
Y. lipolytica	Functional overexpression of four genes including *TKL1*, *TAL1*, *ZWF1*, and *GND1*	Shake-flask experiment	51.09	0.58	0.81	Mironczuk, Biegalska, and A. Dobrowolski (2017)
Y. lipolytica	Disruption of YALI0F01606g	Batch bioreactor	35.7	0.49	0.59	Carly et al. (2017a, 2017b)
Y. lipolytica Wratislavia K1	Addition of Span 20 surfactant	Fed batch culture	142	0.47	1.1	Rakicka et al. (2016)
Y. lipolytica MK1	Ultraviolet mutagenesis	Batch culture	82.2	0.55	0.84	Mirończuk et al. (2015)
Y. lipolytica MK1	Ultraviolet mutagenesis	Repeated batch culture	224	0.77	0.54	Mirończuk et al. (2015)
Y. lipolytica CICC 1675	Osmotic pressure controlled strategy	One-stage fed-batch fermentation	194.3	0.54	0.95	Yang et al. (2014)
Y. lipolytica	Ultraviolet mutagenesis and medium optimization	Shake flask batch culture	39.24	25.06	–	Ghezelbash, Nahvi, and Emamzadeh (2014)
Y. lipolytica	Mineral supplementation (manganese ion)	Bioreactor culture	47.1	0.47	0.87	Tomaszewska, Rywińska, and Rymowicz (2014a) and Tomaszewska, Rymowicz, and Rywińska (2014b)
Y. lipolytica Wratislavia K1	Glycerol medium with 2.5 %NaCl supplementation	Shake flask experiment	80.0	0.49	1.0	Tomaszewska, Rywińska, and Gładkowski (2012)
Y. lipolytica Wratislavia K1	Acetate-negative mutant	Fed-batch cultures	170	0.56	1.0	Rymowicz, Rywińska, and Marcinkiewicz (2009)

tentatively associated with the metabolic pathway of erythritol. Based on the fact that all genes involved in erythritol production are present in *Y. lipolytica* genome, a putative metabolic pathway for erythritol synthesis has been suggested in *Y. lipolytica* (Figure 2).

In the last decade, great progress has been made in developing different metabolic and bioprocess strategies in *Y. lipolytica* to improve the biosynthesis of erythritol as a valuable functional sugar (Table 1). In order to improve the erythritol titer from glycerol, Carly et al. (2017a, 2017b) constructed a set of *Y. lipolytica* derived strains by overexpressing genes producing key enzymes related to the erythritol biosynthesis pathway. The best outcomes were achieved using a mutant with *GUT1* (encoding a glycerol kinase) and *TKL1* (encoding a transketolase) overexpression, and the *EYK1* (encoding erythrulose kinase) disrupted mutant. Notably, the erythrulose kinase is associated with an initial step of the erythritol catabolic pathway. Fermentation results revealed that the resultant metabolically engineered strain showed 75% higher productivity of erythritol as compared to the wild type strain. Additionally, the cultivation duration was reduced by 40% to realize maximal concentration. The inability of strain to erythritol consumption, it had produced further increase the efficiency of the process. Tomaszewska, Rywińska, and Rymowicz (2014a) and Tomaszewska, Rymowicz, and Rywińska (2014b) obtained

superior results using parent *Y. lipolytica* Wratislavia K1 strain in the fed-batch system by a pulsed supplementation of glycerol (325 g/L). Under these conditions, the parent yeast synthesized a high erythritol titer of 201.2 g/L after 168 h of process time, which corresponds to yield and productivity of 0.62 g/g and 1.2 g/(L·h), respectively. An acetate-negative derivative of *Y. lipolytica* Wratislavia K1 possesses the capability to the simultaneous production of high concentrations of citric acid and erythritol in glycerol-based fermentation media (Rymowicz et al. 2006; Rymowicz, Rywińska, and Gładkowski 2008).

Erythrose reductase that executes the final step, exhibits a significant role in erythritol biosynthesis. It catalyzes the reduction of erythrose to erythritol in the presence of NAD(P)H as a cofactor. Janek et al. (2017) described the explicit role of ER for erythritol production in *Y. lipolytica*. For this, the *YALI0F18590*g gene-encoding ER from *Y. lipolytica* was overexpressed that led to an erythritol titer of 44.44 g/L (a 20% increase than the control), which corresponded to a yield of 0.44 g/g and productivity of 0.77 g/L/h. The purified enzyme displayed the utmost catalytic activity at pH 3.0, and 37 °C and the incorporation of Zn^{2+} ions drive up the activity of ER and thereby erythritol synthesis.

The core issue confronted by researchers and biotechnologists in industrial processes is not only reducing production costs but also to improve the biosynthesis performance of the target product with an instantaneous decrease in by-products generation (Mirończuk et al. 2015). It is demonstrated that mutagenesis might be an effective way to boost up the erythritol production in *Y. lipolytica* (Ishizuka et al. 1989; Lee, Song, and Kim 2003; Ryu et al. 2000). With an aim to increase erythritol titer along with minimal by-products formation, ultraviolet (UV) mutagenesis was applied to generate mutants of *Y. lipolytica* Wratislavia K1. One of the best performing mutants namely MK1 was capable of producing up to 82.2 g/L erythritol with a corresponding yield and productivity of 0.55 g g^{-1} and 0.84 g $L^{-1}h^{-1}$, respectively, in the batch culture. Interestingly, the level of by-products was reduced to less than 5.0% of all metabolites synthesized during the process. With regard to the batch culture, the application of repeated batch cultivation (RBC) enables superior synthesis performance of the bioprocess by prolonging the operative production phase. In the RBC, the newly isolated mutant MK1 yielded the highest erythritol titer of 224 g L^{-1} that corresponded to yield and productivity of 0.77 g g^{-1} and 0.54 g $l^{-1}h^{-1}$, respectively. The by-products concentration was further diminished to only 2.3% of all metabolites (Mirończuk et al. 2015). Ghezelbash, Nahvi, and Emamzadeh (2014) also adopted a UV mutation approach to improving erythritol production along with the elimination of glycerol production by *Y. lipolytica*. They constructed a series of different mutants of wild type *Y. lipolytica* DSM70562 by creating alterations in the erythrose reductase pathway following exposure to UV irradiation. Triphenyl tetrazolium chloride (TTC) agar plate assay was used to screen out mutants presenting the highest ER activity. Amongst the mutants generated, one of the mutants designating mutant 49 appeared as the best erythritol synthesizing strain without the production of any byproducts, in particular, glycerol. In contrast to the parent strain, the mutated strain displayed a 60.36% improvement in erythritol productivity in shake-flask cultures under the optimal medium composition of pH 5.42, initial glucose level, 279.45 g/L, and ammonium sulfate of 9.28 g/L. A comparative sequence analysis between the wild and mutant gene sequences revealed that the Asp^{270} amino acid was replaced with Glu^{270} in erythrose reductase protein. The feasibility of the UV mutated *Y. lipolytica* MK1 derivative was also revealed by Rakicka et al. (2017) to enhance the biosynthesis of erythritol on glycerol-based media in a single-step continuous culture. Experimental results achieved 113.1 g/L of erythritol concentration, with yield and productivity of 0.57 g/g and 1.14 g/(L·h) in the feeding medium using an optimized C: N ratio of 80:1. The same strain, *Y. lipolytica* MK1, synthesized up to 82.2 g/L of erythritol from the glycerol medium with yield and productivity of 0.55 g/g and 0.84 g/(L·h), respectively, in batch culture (Mirończuk et al. 2015). Incubation of *Y. lipolytica* Wratislavia K1 with crude glycerol (300 g/L) results in enhanced erythritol titer of 170 g/L with the corresponding yield of 0.56 g/g, while productivity was recorded to be very (1.0 g/(L·h)) in the fed-batch mode (Rymowicz, Rywińska, and Marcinkiewicz 2009).

Y. lipolytica necessitates a high and continuous demand for oxygen, which is conceived as a major hindrance in the scale-up production process. It has shown that overexpressing bacterial hemoglobin from *Vitreoscilla stercoraria* (VHb) can induce cell biomass and supports the biosynthesis of desired metabolites i.e. numerous proteins in various microorganisms (Bhave and Chattoo 2003; Wang et al. 2012). Indeed, VHb overexpression enhances O_2 transfer in the host, resulting in improved aerobic metabolism (Zhang et al. 2007). Dissolved oxygen is a critical factor that has a substantial influence on the morphology and metabolism of *Y. lipolytica* (Bellou et al. 2014). A high aeration level is considered crucial for efficient biosynthesis of citric acid from glycerol-based waste (Kamzolova et al. 2011; Morgunov, Kamzolova, and Lunina 2013). A level of 20–60% is accounted optimum pO2 for citric acid production in ethanol-grown yeasts, whereas a low citric acid titer under low aeration conditions (5.0%) was related to a profound reduction in enzymes activities associated with the TCA and glyoxylate cycle (Kamzolova et al. 2003). Elimination of oxygen-deficient environment led to enhanced biosynthesis of total organic acids in parent and engineered *Y. lipolytica* strains when grown in sucrose medium (Förster et al. 2007). Furthermore, optimal biosynthesis of α-ketoglutaric acid from rapeseed oil (Kamzolova and Morgunov 2013) or ethanol (Kamzolova et al. 2012) was ensured only at high oxygen level. However, it remains unclear how VHb overexpression affects the productivity of *Y. lipolytica* at low pH and high osmotic pressure, particularly when glycerol is used as a carbon source. Elevated glycerol concentration rises osmotic pressure and thus serves as a stress factor (Yang et al. 2014). In a recent study, Mirończuk et al. (2019) overexpressed a codon-optimized VHb in *Y.*

lipolytica yeast to achieve high growth and efficient erythritol biosynthesis from glycerol under low oxygen conditions. It was observed that yeast strain with VHb overexpression showed an 83% greater erythritol production in shake-flask fermentations. In contrast to the native strain (Yield, 0.29 g/g; productivity, 0.30 g/L.h), the genetically modified strain presented higher erythritol yield and productivity of 0.37 g/g and 0.38 g/L.h, respectively, in bioreactor-based experiments. Promisingly, the engineered derivative produced a very small quantity of citric acid, as compared with a previous study (Mironczuk, Biegalska, and Dobrowolski 2017), and mannitol and arabitol titers differed at a concentration below 2 g/L. This high selective ability during the industrial production of the target product is a beneficial aspect since it trims down the excessive purification costs. Moreover, low agitation during the fermentative bioprocesses results in low foam formation, which is a significant challenge in the biotechnology industry.

Reduced pH of the cultivation media is the major factor in the production of erythritol from glycerol by *Y. lipolytica*. In addition, the increased osmotic pressure also appears to have a significant impact (Tomaszewska, Rywińska, and Rymowicz 2014a; Tomaszewska, Rymowicz, and Rywińska 2014b). In this context, Tomaszewska, Rywińska, and Rymowicz (2014a) and Tomaszewska, Rymowicz, and Rywińska (2014b) inspected the effect of divalent copper, manganese, iron, and zinc ions on the activity of ER and erythritol biosynthesis from glycerol by *Y. lipolytica*. Notably, the tested minerals did not exhibit any inhibitory effect on the growth of yeast. Whereas, the addition of $MnSO_4$ resulted in increased erythritol titer by 14.5%, and the erythritol production reached 47.1 g l^{-1} with a volumetric productivity of 0.87 g l^{-1} h^{-1} in the bioreactor culture with supplementation of manganese ion. Incorporation of Mn^{2+} promoted the ER activity up to 24.9 U g^{-1} of dry biomass weight, which corresponds to 1.3-times higher compared with the control.

As can be noticed from the afore-mentioned reports that *Y. lipolytica* is a highly efficient cell factory for erythritol production, its ability to utilizing erythritol as a carbon source undesirably influences the titer and productivity of erythritol. Hence, it is meaningful to isolate or develop a derivative incapable of catabolizing erythritol. By means of insertional mutagenesis, Ficker's group isolated a *Y. lipolytica* mutant that was not able to grow on erythritol. A detailed genomic analysis revealed that the phenotype of the mutant was directly linked to the YALI0F01606g gene disruption. Experimental results proposed that the newly identified gene, renaming EYK1, translates into an erythrulose kinase. The resulting mutant strain presented an elevated erythritol production ability than that to the parent strain. During the cultivation in a bioreactor, it exhibited a 26% and 30% greater yield and productivity compared with the original strain. Furthermore, it also converted erythritol to erythrulose under particular reaction conditions (Carly et al. 2017a, 2017b).

During the erythritol production, some by-product compounds such as D-arabitol, ribitol, citrate, mannitol, glycerol, and fumarate can be secreted by different microbial strains. After the fermentative process, the purification of erythritol by crystallization from the culture supernatants leverages a huge quantity of viscous and reddish-brown liquor referred to as waste erythritol mother liquor (WEML). This waste molasses comprises numerous low-cost organic compounds that are difficult to separate. As one of the major erythritol-producing countries, about 10,000 tons of erythritol accompanied by over 2000 tons of WEML were produced in 2016 in China, leading to environmental pollution and disposal problems (Wang et al. 2017). Many investigations have been carried out to upgrade the worth of this waste by recovering erythritol and a number of other polyols by applying simulated moving bed chromatography. Nevertheless, this technique was not suitable due to very high equipment and operating investment as well as poor separating efficacy. In this perspective, bio-based removal and biotransformation have recently garnered incredible researches interest as a highly promising approach. Biological removal strategy offers the remarkable advantages of pronounced specificity and separation efficiency for the retrieval of value-added compounds from crude sugar waste (Ueda, Shinogi, and Yamaoka. 2006; Yoon, Mukerjea, and Robyt 2003). Our research group has made great progress and established different strategies for the effective recovery of rare functional sugars such as L-arabitol and L-arabinose from waste XML (Cheng et al. 2011a, 2011b; Jiang et al. 2011). For example, recently, we described a highly effective bioprocess for the separation of erythritol from the waste erythritol mother liquor (Wang et al. 2017). First, polyol impurities were detected by HPLC and GC-MS based techniques, and then *C. maltosa* CGMCC 7323 metabolized these impurities for erythritol purification. Findings indicated that purity of the erythritol was substantially improved by newly developed bioprocess and, therefore, is anticipated to exhibit excellent economic advantages in waste mother liquor treatment in an eco-friendlier manner.

Structural entities of D-threitol and its applied aspects

D-threitol is a four-carbon sugar alcohol that is mainly produced by some osmotolerant yeasts (Carly et al. 2018; Carly and Fickers 2018). Threitol is naturally found in meaningful concentration in the edible fungus *Armillaria mellea* and *Upis ceramboides*, where it functions as an anti-freezing agent (Miller and Smith 1975). It has also been recognized in leaf tissue of plant supplemented with L-sorbose (a carbon n + 2 analog of erythrulose) (McComb and Rendig 1963). It is the major end-product of D-xylose metabolism in humans and this bioconversion is thought to occur in the liver (Pitkänen 1977). D-threitol exhibits a wide range of biotechnological applications including green chemistry, pharmaceutical, food, and medicine. It is a noteworthy precursor to manufacture numerous chiral auxiliaries such as treosulfan that is a bi-functional alkylating agent applied to cure patients suffered from ovarian cancer (Köpf-Maier and Sass 1992). It is also utilized to synthesize ter-butyrate ester, an

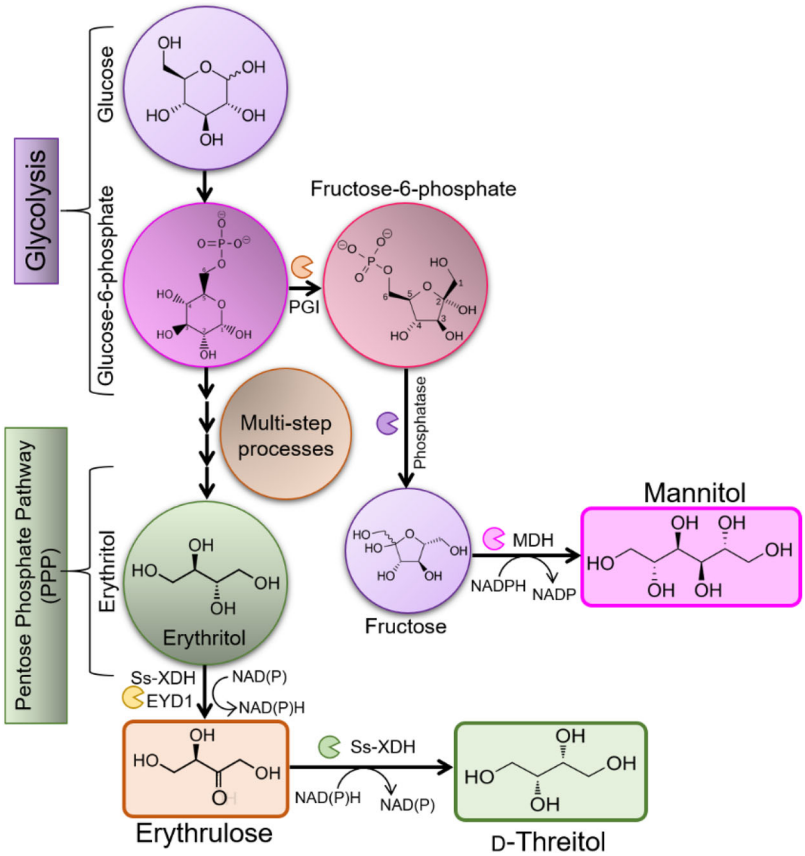

Figure 3. Proposed pathway for threitol synthesis in *Yarrowia lipolytica* strain HC110. Ss-XDH: xylitol dehydrogenase; EYD1: erythritol dehydrogenase; MDH: mannitol dehydrogenase; PGI: 6-phosphate glucose isomerase.

anticancer drug, threitol ceramide, and interleukin-4 and interferon-gamma (Kaur et al. 2011). A range of various artificial amphiphilic phosphates and synthetic phospholipids can be derived from the threitol. It is also an essential component of oxygen-responsive pigment integrated into the smart plastic film that is employed for food packaging (Mills et al. 2012). Keeping in view the increasing applications of threitol, xylitol dehydrogenase gene (Ss-XDH) has been identified and characterized from *Scheffersomyces stipitis* CBS 6054 (Rizzi et al. 1989). Notwithstanding, this enzyme catalyzed the reversible bioconversion of xylitol into xylulose, but it was capable of irreversible oxidation of erythritol to erythrulose followed by its reduction to threitol with a profound efficacy. On this ground, Chi et al. (2019) demonstrated a novel bioprocess to synthesize threitol using glucose as a feedstock via erythrulose intermediate. In order to accomplish this objective, they expressed the Ss-XDH gene into yeast *Y. lipolytica*, which exhibits an efficient ability for erythritol synthesis from glucose. The threitol was then produced directly by the tailored yeast with a titer and yield of 112 g L^{-1} and 0.37, respectively, from the glucose substrate. However, unexpected upregulation of gene encoding mannitol dehydrogenase in this strain results in the buildup of mannitol in the culture broth. To eliminate the mannitol byproduct and erythritol coproduction, a novel wild-type *Candida parapsilosis* based bio-removal method was proposed to drive up the subsequent production and purification of threitol sugar. Figure 3 depicts a proposed pathway for the biosynthesis of threitol in *Y. lipolytica* strain HC110.

Fructooligosaccharides – characteristics and applications

Fructooligosaccharides (FOS) are oligosaccharides that naturally occur in plants, and consist of straight chains of fructose monomers connected through $\beta(2-1)$ linkages. These oligosaccharides possess numerous fascinating properties such as calorie-free, non-cariogenic and low sweetness intensity, and are perceived as soluble dietary fibers. The prebiotic effect, low carcinogenicity, high mineral absorption and ability to diminish concentrations of serum tri-acylglycerols, cholesterol, and phospholipids are the additional valuable physiological effects of FOS. Presently, FOS has found increasing use in infant formulas and food products owing to their prebiotic effect that promotes the development of innocuous intestinal microbial flora (Sabater-Molina et al. 2009). A dietary intrusion of FOS prebiotics has been found beneficial to treat human obesity and Prader-Willi syndrome (Xiao et al. 2014). After administration, the number of endotoxin-producing bacteria was reduced in the gut of obese human hosts, along with enrichment of beneficial bacterial communities, leading to a substantial alleviation of adiposity, inflammation, and insulin resistance (Pokusaeva, Fitzgerald, and van Sinderen 2011). These unique features

Table 2. Comparison of yields and productivity levels of fructooligosaccharides from different bioprocesses using various fructosyltransferase.

Source of fructosyltransferase	Mode of production processes	Reaction duration (h)	Yield (%)	Productivity (g L^{-1} h^{-1})	References
Engineered *Yarrowia lipolytica*	One-stage process using free cells	3	60	160	Zhang et al. (2016)
Aspergillus japonicus ATCC 20236	Two-stage process using FTase	20	87	10.44	Mussatto et al. (2013)
Aspergillus pullulans	One-stage process using free cells	48	64.1	2.06	Dominguez et al. (2012)
Aspergillus japonicus ATCC 20236	One-stage process using cells immobilized on coffee silver skin	16	61 – 70	8.05	Mussatto and Teixeira (2010)
Penicillium expansum MUM 02.14	One-stage process using free cells	36	58	3.25	Prata et al. (2010)
Aspergillus sp. N74	One-stage process using free cells	5.3	50-53	–	Sánchez et al. (2010)
Aspergillus japonicus ATCC 20236	One-stage process using immobilized cells on corncobs	21	66	6.61	Mussatto et al. (2009)
Aspergillus sp. N74	One-stage process using free cells	4	70	122.5	Sánchez et al. (2008)
Aspergillus oryzae CFR 202	Two-stage process using extracellular enzyme	18	53	17.6	Sangeetha, Ramesh, and Prapulla (2005)
Aspergillus oryzae CFR 202	Two-stage process using FTase immobilized on corn germ	8	60	45	Sangeetha, Ramesh, and Prapulla (2004)
Penicillium citrinum	One-stage process using whole wet cells	24	55	–	Hayashi et al. (2000)
Aspergillus niger AS 0023	Two-stage process using purified FTase	54	5.0	–	L'Hocine et al. (2000)

make FOS a noteworthy food component in milk formulas, yogurts, and baking products.

Currently, FOS production involves the synthesis of fucosyltransferase (FTase) by the cultivation of the whole cells (*A. niger*, *A. oryzae*, *A. pullulans*, *A. japonicus*, etc.), enzymes purification from the culture media, and the acquaintance of FTase to its substrate sucrose. These strategies for the production of FOS are laborious, expensive and time-consuming. The use of whole-cells for one-pot production of FOS in bioreactors is a good choice because it circumvents the requirement for FTase enzymes purification from the culture medium (Jung et al. 2011; Sánchez et al. 2008). Irrespective of methods used, the production of FOS is always accompanying high levels of low-value byproducts including fructose and glucose that are necessary to separate for yielding a high purity FOS. Therefore, the development of an FTase-producing microorganism with the FTase enzyme displayed on the cell surface is an important way of converting glucose to industrially pertinent polyols. In this avenue, Zhang et al. (2016) reported a sustainable and cost-efficient approach for the synthesis of FOS using erythritol-synthesizing yeast cells. Immobilization of *A. oryzae* derived FTase on the cell surface of *Y. lipolytica* yielded an engineered strain that produced 480 g/L FOS within 3 h at pH 6.0 and 60 °C. Due to the elevated stability of cell surface-displayed FTase, the whole-cell catalysts can be recycled to at least 10-times, while retaining 90% of its original FTase enzyme activity. The yield and productivity are considered as the two most imperative parameters for the industrial production of FOS. The maximum yield and productivity of FOS obtained was 60% and 160 g/L·h. In another report, Mussatto et al. (2013) achieved the highest FOS yield of 87% by a two-stage bioprocess using FTase produced from SSF of *A. japonicus*, however, the productivity of FOS only reached 10.44 g/(L·h). Biosynthesis processes that enable FOS with high yield and productivity levels are thought to have promising industrial applications because of the lower operational and capital costs. A comprehensive comparison of FOS production yields and productivity levels from different bioprocesses using different FTases is listed in Table 2.

Biosynthesis and biological functions of galactooligosaccharides

Galactooligosaccharides (GOS) is made up of various galactosyl residues (from 2 to 9 units) and terminal glucose joined by β-glycosidic linkages. Naturally, GOS is present at very low concentrations in the milk of animals and humans (Barile and Rastall 2013) but it can also be synthesized by chemical glycosylation or biocatalytic processes (Contesini et al. 2019). In recent years, GOS have received high global demand owing to their health-promoting beneficial effects, such as alleviation of inflammation, reducing risk of colon cancer, decreasing the enteropathogens invasion, improved host immunity, and enrichment of bifidobacteria (Bruno-Barcena and Azcarate-Peril 2015; Davis et al. 2011; Searle et al. 2010). Introducing GOS into food products is highly beneficial in dairy products and infant formula feeds and can imitate the biological functionalities of human milk oligosaccharides (Torres et al. 2010).

A four-step process including enzyme production, purification, immobilization, and transformation is currently used for the synthesis of GOS, which is expensive and time-consuming. Industrial GOS production entails the application of the β-galactosidase enzyme that presents the glycoside hydrolase as well as galactosyltransferase activity. This enzyme has been purified and characterized from culture extract of different strains such as *A. oryzae*,

Table 3. Comparison of galactooligosaccharides production by different β-galactosidases in various modes of process.

Source of enzyme	Mode of production process	Lactose concentration (g L^{-1})	Production (g L^{-1})	Yield (g/g)	Productivity (g L^{-1} h^{-1})	References
Aspergillus oryzae	Cell surface displayed enzyme	500	160	0.32	26.6	An et al. (2016)
Aspergillus oryzae	Free enzyme	500	145	0.29	14.5	Vera et al. (2012)
Saccharolobus solfataricus	Free enzyme	50	14.8	0.29	14.8	Song et al. (2011)
Saccharolobus solfataricus	Free enzyme	600	315	0.52	5.6	Park et al. (2008)
Aspergillus oryzae	Free enzyme	270	54	0.20	108	Matella, Dolan, and Lee (2006)
Aspergillus oryzae	Immobilized enzyme	400	108	0.26	21.6	Albayrak and Yang (2002)
Kluyveromyces lactis	Free enzyme	230	51	0.22	12.8	Foda and Lopez-Leiva (2000)
Aspergillus oryzae	Free enzyme	380	118	0.31	23.6	Iwasaki, Nakajima, and Nakao (1996)

Bifidobacterium, *Lactobacillus reuteri*, *Bacillus circulans*, *Kluyveromyces marxianus*, and *K. lactis* (Gosling et al. 2009; Rodriguez-Colinas et al. 2012; Splechtna et al. 2006; Urrutia et al. 2013). The resultant purified enzymes can either be employed in the free or immobilized form on the carrier's matrix. In contrast to the free enzyme, immobilized biocatalysts offer superior benefits such as stability, continuous operation, reputability, and product purity, and thus substantially decreasing the bioprocessing cost. Nevertheless, immobilized β-galactosidase exhibits the limitations of contamination of support material, enzyme diffusions, and loss of activity (Albayrak and Yang 2002). These facts necessitate the development of cost-efficient and easy-to-operate processes for GOS production. An et al. (2016) demonstrated an interesting strategy for the synthesis of GOS by using the surface-displayed technique in erythritol-producing yeast. *Y. lipolytica* strain was engineered by combining the β-galactosidase gene from *A. oryzae* to the YlPir1 gene that produces a cell wall protein. Results showed that the β-galactosidase was efficiently displayed on the cell surface of engineered *Y. lipolytica* CGMCC7326 strain, which was capable of efficiently producing GOS from lactose. The titer of GOS reached 160 g/L with a corresponding yield of 51% within 6 h using 500 g/L lactose solution at a pH 5.5 and 60 °C. In contrast to the free enzyme, the surface-displayed biocatalyst was observed to be more stable at elevated temperatures and maintained 75% of its catalytic activity after incubated at 75 °C for 1.5 h. On the other hand, the activity of the free enzyme drastically reduced to 60 °C (Albayrak and Yang 2002). The A.oryGal can be recycled in several repeated cycles in the immobilized form using a surface-displayed expression system. After 10 repeated cycles, the enzyme was capable of retaining 85% of the original activity that diminished to 65% in the 15th continuous cycle. Though the immobilized form of β-galactosidases has been extensively utilized to produce GOS production (Gaur et al. 2006; Matella, Dolan, and Lee 2006), the cell surface display technology has been regarded a promising strategy for the efficient synthesis of GOS in repeated biotransformation. As compared to enzyme immobilization on carrier supports, the yeast surface-displayed enzyme system exhibits numerous advantages. It can substitute the strenuous process of protein purification and alleviates the sorbent requirement for enzyme immobilization. Reports have shown that a wide range of enzymes have been effectively displayed on the surface of the yeast cell and presented superior catalytic activities than that to commercial enzymes or secreting strains (Duquesne et al. 2014; Yamakawa et al. 2012). The comparative evaluation of GOS production by various β-galactosidases in different process modes is summarized in Table 3.

Isomaltooligosaccharide – functional entities

Isomaltooligosaccharide (IMO) is a mixture of glucose oligomers that contain one or several $\alpha-1,6$ as well as $\alpha-1,4$ glycosidic linkages. These carbohydrates included panose, isopanose, isomaltose, isomaltotriose, isomaltotetraose, isomaltopentaose, and other longer chain oligosaccharides (Goffin et al. 2010). In addition to extensive application in the food industry, IMO has also been utilized in cosmetics and medical industries. As a good prebiotic, IMO play a noteworthy role in improving probiotic community in the organism's digestive tract, facilitating the minerals absorption, maintaining a robust micro-ecosystem, regulating the triglyceride and cholesterol levels, and boosting up the immunologic functionalities of the body (Florowska et al. 2016; Panesar, Kumari, and Panesar 2013; Swennen, Courtin, and Delcour 2006; Yen et al. 2011). Owing to these interesting physiological functions and physicochemical properties, IMO are associated with enormous consumption in foods such as in ferments, dairy products, yellow wine, and functional beverages. Characteristic methods for IMO to synthesis implicate a multi-enzymatic system using the maize starch as a raw material. For this, emulsified maize starch is transformed into maltodextrin with the aid of a mixture of α-amylases. Afterward, fungal pullulanase and β-amylase are applied to maltodextrin saccharification. The enzyme-treated saccharified mixture comprises over 50% maltose, which is then transglycosylated by *Aspergillus*-derived α-transglucosidase at 50 °C to yield IMO (Kim et al. 2003). This procedure is laborious and cumbersome necessitating many reaction steps as well as a longer duration to produce IMO. Furthermore, the preparation of the enzymes through fermentation for IMO biosynthesis with starch is

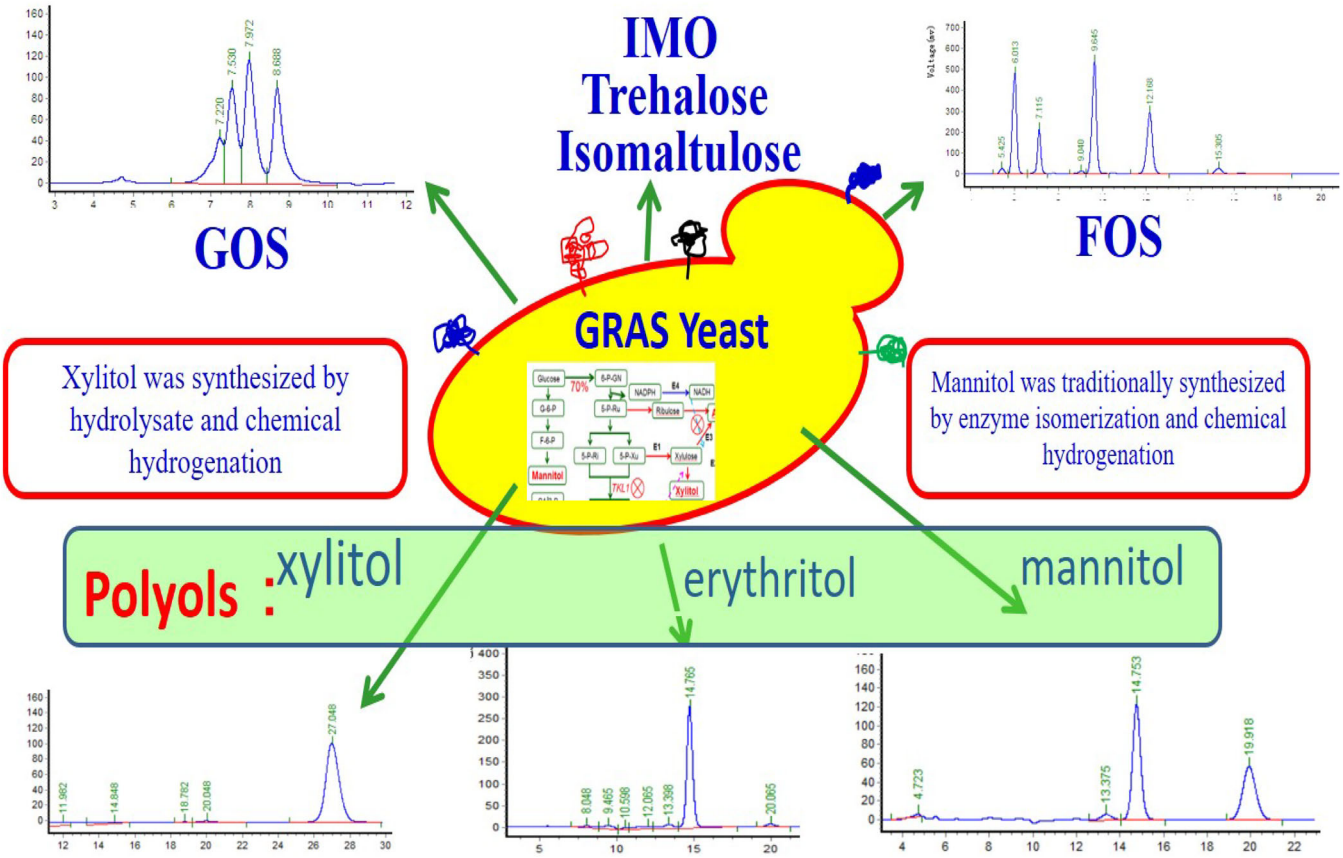

Figure 4. Cascade synthesis technology for the biosynthesis of polyols and oligo-saccharides.

expensive and time inefficient. Therefore, the synthesis technique without the requisite of the purified enzyme and shorter bioconversion time is significantly helpful to reduce the production cost. To overcome this issue, our group recently developed a cheap and easy-to-use method by employing a food-safe *Y. lipolytica* strain. The genes encoding α-transglucosidase and β-amylase were merged and allowed to co-display on the surface of this yeast cells. The as-engineered strain can efficiently catalyze the conversion of liquefied maize starch into IMO in a one-step process. The maximum purity of produced IMO was recorded to be 75.3% by using the co-displayed fused enzyme system at 50 °C (Liu, Cheng, and Deng 2019). Figure 4 presents a cascade biosynthesis technology for the production of oligo-saccharides

Isomaltulose – functional features and applications

Isomaltulose (palatinose) is a disaccharide consist of D-glucose and D-fructose connected by the $\alpha-1,6$-glycosidic bond. Isomaltulose is a structural sucrose isomer with identical organoleptic and physical characteristics to those of sucrose. Nevertheless, it presents enormous benefits to sucrose. It is a non-cariogenic nutritive sugar with a low-caloric content because of less sweetness compared with sucrose. Furthermore, isomaltulose has great potential to serve as the best sucrose substitute for diabetic and obese individuals (Li et al. 2017). Traditionally, industrial-scale isomaltulose is produced by a two-stage microbial process, in which enzyme (sucrose isomerase) is first synthesized by microbial fermentation and then used to catalyze an enzymatic reaction with a suitable substrate for isomaltulose biosynthesis (Goulter, Hashimi, and Birch 2012; Wu et al. 2015). It can also be produced by whole-cell transformation based on a single-step bioprocess, where the enzyme synthesis and catalytic reaction are carried out in a single fermentation process (Kawaguti and Sato 2010). The whole cell mediated biotransformation circumvents laborious sucrose isomerase purification from cell extracts owing to the utilization of whole cells as a biocatalyst and thus facilitating the production of isomaltulose production in a single step. A range of bacterial strains including *Klebsiella sp. LX3*, *Erwinia rhapontici*, *Pantoea dispersa*, and *Serratia plymuthica* have been exploited for the whole-cell catalyzed bioconversion of sucrose to isomaltulose (Kawaguti and Sato 2010; Li et al. 2011; Wu and Birch 2004). Carrier-immobilized cells have also been considered because of the relative easy cell recovery from the fermented broth and profound resistance capacity to elevated levels of substrates and products (Kawaguti and Sato 2007). A yeast surface display technology has recently been proposed as a prevailing molecular approach for protein engineering and was used to transform sucrose to isomaltulose, though very low yields of only 6.4–7.4% (Lee et al. 2011). Li et al. (2017) demonstrated the successful display of *P. dispersa* derived sucrose isomerase on the cell surface of *Y. lipolytica* in the presence of cell wall protein Pir1 as an anchor protein. The as-engineered sucrose isomerase led to the conversion of isomaltulose with

a maximum yield of 93%. The enzyme displayed yeast derivative showed stability in a broader pH (ranging from 4.5 to 7.0) and temperature ranges (ranging from 20 to 40 °C). In addition, none of the glucose or trehalose byproducts was identified during the biotransformation process, and the engineered yeast cells presented viability for up to 12 continuous batch operations cycles, retaining conversion efficacy of more than 80%.

Tai and Stephanopoulos (2013) characterized a newly developed strong constitutive promoter namely TEF promoter with an intron (TEFin), which is capable of inducing a 5-fold increased expression in *Y. lipolytica* in comparison with the traditional promoter. The production of isomaltulose using sucrose isomerase-mediated catalytic methods were thought to more suitable but were hindered because of low enzyme activity and lack of sufficient SIase secretion. In order to achieve efficient secretory SIase expression levels, Zhang et al. (2019) carried out the overexpression of a SIase gene from *Pantoea dispersa* into *Y liolytica* host using TEFin as a strong constitutive promoter. The resultant modified strain results in the secretion of high activity of SIase with (49.3 U/mL) under the optimal culture medium conditions. Effective immobilization of the purified recombinant SIase onto the polyvinyl alcohol-alginate matrix showed a prominent enzyme recovery of more than 80%. After immobilization, the stable SIase enzyme catalyzed the generation of 620.7 g/L isomaltulose with a corresponding yield of 0.96 g/g in optimized batch production conditions. The immobilized biocatalytic system presented high recyclability retaining above 90% of sucrose bioconversion efficiency after 13 consecutive batches. The findings revealed the SIase expression and immobilization as a promising approach for the large-scale biosynthesis of isomaltulose.

Structural and functional aspects of trehalose

Trehalose is a non-reducing disaccharide, in which two glucose molecules are connected through $\alpha,\alpha-1,1$-glycosidic bond. It is one of the most efficient molecules that provide protection to the cells against stress and preserves membrane and protein integrity in desiccation by the replacement of water with osmolyte molecules (Zheng et al. 2015). Trehalose has gained wider applications in the pharmaceutical, agri-food, cosmetic and other industries because of its fascinating properties that are shared by robust stability and most chemically inert sugars (Richards et al. 2002; Schiraldi, Di Lernia, and De Rosa 2002). Though these qualities have been acknowledged from last several years, trehalose has not been synthesized on a commercial scale for a long time. Recently, two enzyme-based procedures were applied commercially to synthesize trehalose. In the first method, maltooligosaccharides or starch or were utilized to produce trehalose by maltooligosyltrehalose hydrolase and maltooligosyltrehalose synthase catalyzed reactions in amalgamation with α-amylase and pullulanase (de Pascale et al. 2002; Fang et al. 2006). In the second method, trehalose was directly synthesized from maltose by trehalose synthase (TreS) via intramolecular transglycosylation (Chang et al. 2010; Zheng et al. 2015). Amongst these methods, one-step TreS-catalyzed conversion of maltose into trehalose through intramolecular reorganization is a fast, simple, and less expensive method and presents great promise for scalable trehalose biosynthesis. A large number of *treS* genes from various bacterial strains have been isolated, characterized, and applied in the synthesis of trehalose (Chen, Lee, and Shaw 2006; Jiang et al. 2013; Kim et al. 2010; Wu et al. 2009), but the conversion yield of trehalose from maltose by *treS*-containing bacteria was very low. Thus, the yield was improved by cloning *treS* genes from those bacteria, overexpression in *E. coli*, and using as biocatalysts for conversion of maltose to trehalose (Chen, Lee, and Shaw 2006; Kim et al. 2010). This synthesis method involves the cultivation of *E. coli* in an antibiotic-incorporated medium followed by IPTG induction, TreS purification from the crude extract and then maltose transformation to trehalose by using enzyme either in free or immobilized form. Consequently, these methods are not only complicated and laborious but are also not affordable, particularly in essence of enzyme purification. In a recent study, Zheng et al. (2015) demonstrated the production of trehalose from maltose by using robust whole cells of permeabilized recombinant *E. coli* and achieved a high titer of 92.2 g/L with the productivity of 23.1 g/L.h under the optimized processing conditions. Our group developed a low-cost and simple alternative strategy for the biosynthesis of trehalose from maltose (Li et al. 2016). To this end, the TreS enzyme obtained from the *P. torridus* was surface-immobilized on the *Y. lipolytica* cell and subsequently applied as a whole-cell biocatalyst for direct bioconversion of maltose to trehalose. Under optimal conditions, the yield of trehalose reached 73%, and the pH and thermal stability profile of recombinant biocatalyst were substantially improved than that to the free enzyme from *E. coli*. After the completion of the biotransformation process, the residual maltose and glucose byproducts were directly fermented to ethanol by adding *S. cerevisiae* strain, and the resulting ethanol was separated to obtain highly pure trehalose. This *Y. lipolytica* surface display based newly proposed a one-pot consolidated method is a promising approach to the cost-effective synthesis of trehalose from maltose disaccharide, since it eliminates the requisite of enzyme purification and immobilization, and thus remarkably diminishing the process costs.

Biosynthesis of erythrulose for the biomedical sector

Erythrulose is a tetrose carbohydrate that can be used as a useful precursor to produce glyceraldehyde acetonide (De Wilde et al. 1987). This compound might have potential application for the preparation of a range of bioactive molecules such as Tanikolide (an antifungal compound), Bengamide E (an anticancer drug), cytoxazone (the cytokine modulator), and substituted β-lactams (Arasaki et al. 2004; Metri, Schiess, and Prasad 2013; Miranda et al. 2016; Wagle et al. 1988). It served as an excellent precursor in the biosynthesis of L-erythrose, cholesterol-reducing drugs (Zetia

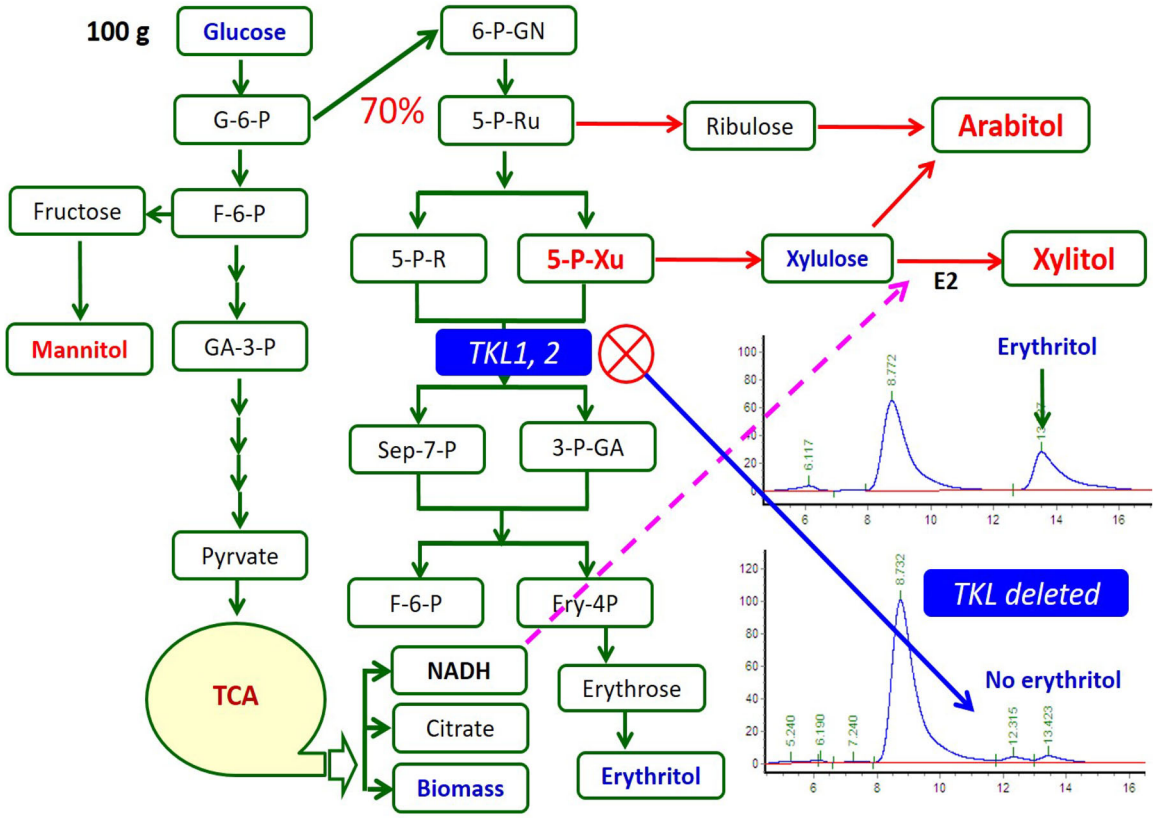

Figure 5. Schematic illustration of engineered *Yarrowia lipolytica* for functional sugar, alcohol erythritol, mannitol, and xylitol production.

and Crestor), chiral amino alcohols, and the hypotensive antiepileptic drug (γ-amino-8-hydroxybutyric acid) (Matosevic, Lye, and Baganz 2011; Mizanur et al. 2001; Van der Eycken et al. 1987). It also finds uses in cosmetic products as a sunless tanning agent. Like dihydroxyacetone, erythrulose reacts with skin's keratin through Maillard reactions resulting in tawny effects. Though dihydroxyacetone is currently the most prevalent sunless-tanning compounds, it generates an unusual yellow-orange shade (Jermann, Toumiat, and Imfeld 2002). Whereas, erythrulose ensures natural, homogeneous, and long-lasting skin tone and overcomes the aforementioned known limitation of dihydroxyacetone such as skin dryness and red streaking.

Erythrulose can be produced from hydroxypyruvate and glycolaldehyde using the purified transketolase from *E. coli*. The process can be accomplished by using the free form of the enzyme, enzymes incorporated microreactors or microfluidic enzyme reactors (Matosevic, Lye, and Baganz 2011; O'Sullivan et al. 2012). Erythrulose can also be generated from the oxidation of erythritol by microbial fermentation. Due to the increasing demand of erythrulose, new and efficient bioconversion processes have been developed using various bacterial strains such as *Gluconobacter frateurii*, *G. oxydans*, and *G. kondonii* with high conversion efficiency, and productivity (Mizanur et al. 2001; Pan et al. 2016). Recently, Carly et al. (2017a) reported the capability of *Y. lipolytica* to synthesize erythrulose from erythritol and secretion into the culture medium. Inactivation of gene YALI0F01606g (*EYK1*) encoding an erythrulose kinase circumvents subsequent erythrulose catabolism, and thus promoting the buildup of erythrulose in the medium. However, the erythrulose accumulation was found to be inadequate due to the lower expression of the gene encoding erythritol dehydrogenase. To address this issue, Carly et al. (2018) isolated and characterized a gene YALI0F01650g (renamed *EYD1*) that encodes an erythritol dehydrogenase. Constitutive expression of *EYD1* in a *Y. lipolytica* mutant with an inactivated *EYK1* gene (encoding erythrulose kinase) yielded a cell factory resulting in the synthesis of erythrulose from erythritol with a bioconversion yield and productivity of 0.64 g/g and 0.116 g/gDCW.h, respectively, in medium containing a low concentration of glycerol.

Biosynthesis and biological role of mannitol

Mannitol is a kind of six-carbon sugar alcohol with numerous applications in the pharmaceutical, medical, and food industries. This polyol is ubiquitously present in nature, including algae, plants, fungi, and fresh mushrooms. Current mannitol synthesis in the industrial level is executed by a chemical method using hydrogenation of fructose at elevated pressure and high temperature, which is not very proficient requiring highly pure substrates (Tomaszewska, Rywińska, and Gładkowski 2012). Thus, the microbial fermentative process is might considered as an interesting alternative for mannitol production (Saha and Racine 2011; Song and Vieille 2009). It is recognized that some bacteria and osmophilic yeast-like fungi, and *Y. lipolytica* yeast has

Table 4. Summary of price, recent manufacturers, and market analysis of different functional sugars.

Functional sugars	Manufacturers	Raw materials	Total production in 2019 (1000 kg)	Prices (USD per 1000 kg, approximate)	Country
Erythritol	Cargill Inc	Glucose	50,000	2500	USA
	Cerestar				Belgium
	Baolingbao Bio.				China
	Sanyuan Bio.				China
Mannitol	Lianmeng Group	Glucose	100,000	2800	China
	Jie Jing Group				China
	ICI, Inc				USA
	Roquettc				France
Xylitol	Huakang Ltd.	Corncob, Xylose	150,000	3500	China
	Linglive				China
	Danisco (DuPont)				USA
	Roquettc				France
FOS	Baolingbao Bio.	Sucrose	30,000	4000–4500 depending on purity	China
	QHT	Sucrose			China
	Faninon	Chicory root, Jerusalem artichoke			China
GOS	Baolingbao Bio.	Lactose	25,000	5000–5500 depending on purity	China
	QHT				China
	Friesland Campina				Netherland
	Yakult				Japan
	Nissin Sugar				Japan
IMO	Baolingbao Bio.	Starch	120,000	1500–1700 depending on purity	China
	Bai Long Chuan Yuan				China
	Showa Denko				Japan
	BioNeutra				Canada
Isomaltulose	Hong Tao Bio.	Sucrose	8000	1800	China
	Haiyi Bio.				China
Trehalose	Meihua Group	Starch	30,000	2000	China
	Fuyang Biotech				China
	Hayashibara				Japan

Data were retrieved from market survey from trade departments of manufacturers.
FOS: fructooligosaccharides; GOS: galactooligosaccharides; IMO: isomaltooligosaccharide

shown the ability to producing polyols or their derivatives under elevated external osmotic pressure (Kayingo, Kilian, and Prior 2001; Veiga-Da-Cunha et al. 1992). The probable use of *Y. lipolytica* is of great interest in polyols production because of its capability to grow in the presence of a high level of NaCl in the medium (Andreishcheva et al. 1999). Onishi and Suzuki (1968) inspected the influence of various substrates on the biosynthesis of mannitol by various yeasts. Among these, glucose appeared to be the most appropriate source of carbon for mannitol biosynthesis in the shake-flasks experiment. Under optimal conditions of 20% glucose addition to the medium, the yeasts were able to produce higher than 44 g L^{-1} mannitol, which relates to a yield of 0.30 g g^{-1}. Importantly, a substantial concentration of mannitol was recorded in cultures with pure glycerol lacking NaCl in the UV mutants (Tomaszewska, Rywińska, and Gładkowski 2012). The titter of mannitol in UV mutant (A UV'1 strain) reached 27.6 g L^{-1} with yield and productivity of 0.16 g g^{-1} and 0.42 g L^{-1} h^{-1}. However, the incorporation of salt to the medium results in a 3-fold reduction in mannitol production. A mannitol titer of 23 and 12 g L^{-1} was obtained in the glucose and glycerol media, respectively, using the Wratislavia K1 (Rymowicz, Rywińska, and Marcinkiewicz 2009). Various strains of *C. magnoliae* synthesized 211 g L^{-1} of mannitol in the fed-batch cultures with optimal fructose-containing media (Lee, Song, and Kim 2003). Figure 5 illustrates a schematic demonstration of the engineered *Y. lipolytica* for functional sugar, alcohol erythritol, and mannitol production.

Concluding remarks and future outlook

The studies on the biosynthesis of valuable functional sugars have emerged as a popular research area owing to their incredible applications in various industrial domains. Nevertheless, their chemical organic means involve multiple reactions steps that are expensive and cumbersome with lower yields. On the other hand, bio-catalytic routes are often associated with elevated catalyst costs and low space-time yields, and discovering a specific enzyme to act as a catalyst for specific sugar is also a major biotechnological challenge in producing these sugars. With the increasing demand for bio-renewable and more sustainable products, microbial conversion has become the major and eco-friendly route for biochemical synthesis. Table 4 summarizes the prices, recent manufacturers, and market analysis of different kinds of functional sugars. *Y. lipolytica* offers several distinctive metabolic advantages such as high flux through the TCA cycle, which constitutes it a supremely promising microbial chassis for a range of biochemical production including nutraceuticals, organic acids, oleochemicals, and many other commodity chemicals. Similarly, industrially pertinent assets including the high tolerance to various chemicals, malleable metabolic regulation, and elevated protein biosynthesis and secreting capacities make *Y. lipolytica* a desirable host. Though considerable progress has been made in this direction, however biotransformation efficiency can be further enhanced by developing new genetic tools and technologies, creating models that are more descriptive, and utilization of state-of-the-art CRISPR/Cas9 system.

These strategies might enable the exploitation of other oleaginous yeasts such as *Rhodosporidium toruloides, Lipomyces starkeyi*, and *Trichosporon oleaginosus*. Though many engineering challenges left to be addressed, the rapid implementation of *Y. lipolytica* both as academic and industrial levels emphasized that this microbial host will grab new opportunities for large-scale bio-production beyond a simple lipid producer in the upcoming years.

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