Genome-scale model of *Rhodotorula toruloides* metabolism

Ievgeniia A. Tiukova¹² | Sylvain Prigent³ | Jens Nielsen¹ | Mats Sandgren² | Eduard J. Kerkhoven¹

¹Systems and Synthetic Biology, Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
²Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden
³UMR 1332 BFP, Univ Bordeaux, Villenave d’Ornon, France

**Correspondence**
Eduard J Kerkhoven, Systems and Synthetic Biology, Department of Biology and Biological Engineering, Chalmers University of Technology, SE-41296 Gothenburg, Sweden. Email: eduardk@chalmers.se

**Funding Information**
Svenska Forskningsrådet Formas, Grant/Award Numbers: 2016-00767, 213-2013-80; Åforsk Foundation, Grant/Award Number: 16-655; Novo Nordisk Foundation, Grant/Award Number: NNF10CC1016517

**Abstract**
The basidiomycete red yeast *Rhodotorula toruloides* is a promising platform organism for production of biofuels. We present *rho*-GEM, the first genome-scale model (GEM) of *R. toruloides* metabolism, that was largely reconstructed using RAVEN toolbox. The model includes 852 genes, 2,731 reactions, and 2,277 metabolites, while lipid metabolism is described using the SLIMEr formalism allowing direct integration of lipid class and acyl chain experimental distribution data. The simulation results confirmed that the *R. toruloides* model provides valid growth predictions on glucose, xylose, and glycerol, while prediction of genetic engineering targets to increase production of linolenic acid, triacylglycerols, and carotenoids identified genes—some of which have previously been engineered to successfully increase production. This renders *rho*-GEM valuable for future studies to improve the production of other oleochemicals of industrial relevance including value-added fatty acids and carotenoids, in addition to facilitate system-wide omics-data analysis in *R. toruloides*. Expanding the portfolio of GEMs for lipid-accumulating fungi contributes to both understanding of metabolic mechanisms of the oleaginous phenotype but also uncover particularities of the lipid production machinery in *R. toruloides*.

**Keywords**
genome-scale model, metabolism, *Rhodotorula toruloides*, yeast

**1 INTRODUCTION**

*Rhodotorula toruloides* (syn. *Rhodospirillum toruloides*) is a basidiomycetous yeast belonging to the subphylum *Pucciniomycotina* and occurs naturally in a wide range of habitats including surfaces of leaves, soil, and sea water (Sampaio, 2011). The broad substrate range of *R. toruloides* and its ability to accumulate lipids exceeding half of its cell dry weight has made this yeast a popular system for the production of pharmaceuticals of industrial relevance including value-added fatty acids and carotenoids, in addition to facilitate system-wide omics-data analysis in *R. toruloides*. Expanding the portfolio of GEMs for lipid-accumulating fungi contributes to both understanding of metabolic mechanisms of the oleaginous phenotype but also uncover particularities of the lipid production machinery in *R. toruloides*.

One of the major determinants of the oleaginous phenotype of *R. toruloides* is its capacity for acetyl-CoA production (Zhu et al., 2012). Unlike non-oleaginous yeasts such as the baker’s yeast *Saccharomyces cerevisiae*, *R. toruloides* possesses the enzyme ATP:citrate lyase, which is the main source of acetyl-CoA for lipid synthesis. In addition, a mitochondrial β-oxidation pathway provides additional source of acetyl-CoA in this yeast (Vorapreeda, Thammarongtham, Cheevadhanarak, & Laoteng, 2012). Lipid biosynthetic reactions downstream of acetyl-CoA synthesis do not differ between oleaginous and non-oleaginous yeast species, simplifying the use template models from non-oleaginous yeasts.
2 | MATERIALS AND METHODS

2.1 | Draft model reconstruction

The genome-scale model, named rhto-GEM, is based on the genome sequence of *R. toruloides* strain NP11 (Zhu et al., 2012). The reconstruction of rhto-GEM was primarily performed using RAVEN 2.2.1, a MATLAB toolbox for genome-scale model reconstruction (H. Wang et al., 2018). All steps of the reconstruction are documented in detail on the GitHub repository under the folder `ComplementaryScripts/reconstruction`. More information on the GitHub repository is provided below.

To reconstruct those parts of the metabolism that are relatively conserved between fungal species, the well-curated GEMs of *Saccharomyces cerevisiae* (yeast-GEM version 8.2.0, https://doi.org/10.5281/zenodo.1495483; Lu et al., 2019) and *Yarrowia lipolytica* (Yali4.1.1; Kerkhoven, Pomraning, Baker, & Nielsen, 2016) were taken as template models, while orthologous genes were identified via bidirectional BLASTP (Camacho et al., 2009) against the *S. cerevisiae* S288c and *Y. lipolytica* CLIB 122 reference genomes. All 1–1 orthologs were included, after cut-offs of E-value <1e−20; identity >35% and alignment length >150 bp. Additional orthologs between *R. toruloides* and *S. cerevisiae* were identified as provided by MetaPhOrs (Przybysz, Huerta-Cepas, & Gabaldón, 2011), filtered for ortholog pairs with confidence scores of 1 and whose PhylomeDB tree contained at least two members.

2.2 | Gap-filling with Meneco

To obtain a functional model, a gap-filling step was performed to add reactions necessary to produce biomass from the preferred growth medium of *R. toruloides*. To avoid self-producing loops due to stoichiometric inconsistencies, we utilized Meneco 1.5.2 (Prigent et al., 2017) in combination with yeast-GEM (v. 8.2.0) as database of repair reactions. In Meneco, target compounds correspond to metabolites present in the biomass function, while seed compounds are composed of metabolites present in the growth medium, plus some cofactors and metabolites required for FBA growth. If no path exists between seed and target compounds, Meneco proposes one minimal set of reactions (or several minimal sets of same size) coming from a database of reactions to fill the gaps. The sets of seed and target compounds are given in on the GitHub repository under the folder `ComplementaryScripts/menteco`. The seed compounds included uncharged tRNAs as the biomass reaction explicitly represents protein translation as the transfer of amino acids from tRNAs. The union of all proposed completions was included in the draft model, while manual curation was performed to confirm the likelihood of those reactions and to identify their corresponding genes in *R. toruloides*.

2.3 | Lipid metabolism with SLIMEr

Lipid metabolism was described using the SLIMEr formalism, which splits lipids into measurable entities (Sánchez, Li, Kerkhoven, & Nielsen, 2019). To minimize the number of unique lipid species, we inferred from experimental data (Wei, Siewers, & Nielsen, 2017) which acyl chains can be expected at each position: in phospholipids and triacylglycerols (TAGS) the sn-1 position is populated by saturated acyl chains (i.e., 16:0 or 18:0); sn-2 positions by unsaturated acyl chains (i.e., 18:1, 18:2 or 18:3) and sn-3 positions by saturated or monounsaturated acyl chains (i.e., 16:0, 18:0, 18:1). Palmitoleate (16:1) is not modeled as it is only a minor contributor (<5%) to the overall acyl chain distribution (Tiukova et al., 2019). Cardiolipin maturation is further simplified by assuming that the monolysocardiolipin acyltransferase only utilizes phosphatidylcholine with acyl configuration 1–16:0; 2–18:1 as cosubstrate. Consequently, this resulted in 67 SLIME reactions and 1,022 curated reactions in lipid metabolism, instead of 920 and 7,130 reactions if all combinations of the five acyl chains (i.e., 16:0; 18:0; 18:1; 18:2; 18:3) were allowed. To facilitate adjusting the lipid composition in the model by considering measured lipid class and acyl chain distributions, we provide the functions, adjustRhtoBiomass and scaleLipidsRhto, in the `ComplementaryScripts/experimental` folder.
2.4 Further model development and distribution via GitHub repository

The growth- and nongrowth-associated energy requirements were fit to measured glucose uptake rates from continuous cultivations of *R. toruloides* as reported in literature (Shen et al., 2013), and set at 132.7 mmol gDCW⁻¹ and 3.39 mmol (gDCW h)⁻¹, respectively. The biomass composition was modified from yeast-GEM to include *R. toruloides* lipid class and acyl chain distributions, as provided in ComplementaryData/data. The consumeSomething and produceSomething functions from RAVEN were used to ensure there is no net production or consumption of mass by any reaction in the model. The rhto-GEM model is hosted on a dedicated GitHub repository (http://github.com/SysBioChalmers/rhto-GEM). Here, all scripts for model reconstruction are provided, in addition to the model in various file formats, for example, SBML, YAML, TXT, and XLSX, and scripts for performing the simulations detailed in this manuscript. This environment allows for versioning and comparison of the model, reporting and tracking of issues, organization of development, and continuous integration. Memote 0.9.2 is a model test suite (Lieven et al., 2018) used to assess model quality, which is automatically run via Travis CI with each new model release, currently skipping the consistency tests due to their long duration.

2.5 Model simulations

Flux balance analysis was performed with RAVEN toolbox, using constraints on exchange fluxes as specified in the text, while also detailed in the relevant scripts in the ComplementaryScripts folder. All simulations here were performed with rhto-GEM version 1.2.1. Gene essentiality was predicted using singleGeneDeletion from COBRA toolbox 3.0.6 (Heirendt et al., 2017), where growth rates reduced by more than two-thirds were classified as lethal. Reactions significantly affecting TAG biosynthesis were identified using singleRxnDeletion from COBRA toolbox. To predict genetic targets for metabolic engineering, the flux scanning of enforced objective function (FSEOF; Choi, Lee, Kim, & Woo, 2010) implementation of RAVEN was used. Exchange reactions were added for the products of interest, which were optimized with either glucose or xylose as carbon source. We performed FSEOF analysis using triacylglyceride (1–18:0, 2–18:1, 3–18:0) as a representative species for TAG, while this TAG is, further, of interest as a major component of cocoa butter. The slope parameter derived from FSEOF is indicative of how strong each reaction is contributing toward a shift from growth toward production of the target compound, and thereby suggests which reactions are promising targets for overexpression to increase productivity.

3 RESULTS AND DISCUSSION

3.1 Step-wise reconstruction of rhto-genome-scale model

To support the development of *R. toruloides* as promising microbial biocatalyst for oleochemical production, we developed a genome-scale model for *R. toruloides* strain NP11 through a combination of semiautomated reconstruction and manual curation based on literature data (Figure 1a). The reconstruction and curation process is tracked in a publicly accessible Git repository, an environment that allows for open, reproducible, and trackable development and curation of genome-scale models by any member of the research community. Users are encouraged to report issues with the existing model and contribute to the continuous development, while the step-wise reconstruction described here is fully documented in the repository.

Bidirectional protein-blast (Camacho et al., 2009) with RAVEN (H. Wang et al., 2018) querying of the *R. toruloides* and *S. cerevisiae* genomes by either BLAST or the phylogeny-based MetaPhOrs identified, respectively, 628 and 571 pairs of orthologous genes that were annotated to the template yeast-GEM model. In addition, querying the phylogeny-based ortholog repository MetaPhOrs yielded 571 pairs of orthologs model-annotated genes. Complementary to *S. cerevisiae* as a template model, also the GEM of oleaginous yeast *Y. lipolytica* (Kerkhoven et al., 2016) was queried for
orthologous genes, resulting in the identification 22 additional orthologous genes. Combined, this first step of model reconstruction rapidly identified 823 R. toruloides genes connected to 2,152 reactions (Figure 1b), representing parts of metabolism that are well conserved between fungal species.

In the second step of model reconstruction, a large number of nongene-associated reactions, primarily pseudo-reactions, exchange and intracellular transport reactions, were transferred from the template model to the draft reconstruction. While such reactions are required to obtain a functional model, not all the template-derived reactions are required to support growth in the final R. toruloides model. Therefore, unconnected nongene-associated reactions were removed from the model at a late stage of the reconstruction.

In addition to automated template-based reconstruction, it is imperative to curate organism-specific reactions and pathways to obtain a representative and comprehensive model. In the third step of rhto-GEM reconstruction, we included seven reactions from the carotene and torulene biosynthetic pathways (Buzzini et al., 2007), while fatty-acid degradation through mitochondrial β-oxidation introduced 67 further reactions (Zhu et al., 2012). Along with synthesis and degradation pathways of 18:2 and 18:3 fatty acids, the third step of model reconstruction increased the reaction and gene count to 3,362 and 842, respectively.

### 3.2 | Topological-based gap-filling

As the resulting draft model was unable to support the production of biomass, we performed gap-filling as the fourth step in the reconstruction. For this, we utilized Meneco (Prigent et al., 2017), a gap-filling tool that aims to find a topological path in the bipartite graph of reactions and compounds between seed and target compounds. By utilizing yeast-GEM as database of reactions, Meneco proposed that 13 reactions should be added to the model to make it functional. A total of 24 different sets of 13 reactions were identified, the union of these having a size of 19 reactions. As no information was available to select a particular set of reactions among the 24 possibilities, we added all 19 reactions. If any of the newly added reactions were catalyzed by an enzyme (i.e., annotated with a gene in the model), then we assumed that also other reactions that are catalyzed by the same enzyme should be included in the draft model. Through this approach, we added three more reactions. Addition of all 22 reactions sufficed for the model to produce biomass.

In addition to the topological-based gap-filling, manual curation throughout the development process identified a number of additional genes and reactions, resulting in an extended draft model with 3,423 reactions and 875 genes.

### 3.3 | Representation of lipid metabolism

As particular interest on R. toruloides is focused on its oleaginous nature, attention was paid to accurately depict lipid metabolism. Recently, we have developed the SLIMEr formalism for describing lipids in genome-scale models, which splits lipids into measurable entities (Sánchez et al., 2019). This formalism represents the flexibility of lipid metabolism while allowing incorporation of measurements of lipid classes and acyl chain distributions, briefly explained in Section 2, while a detailed analysis of the practical implications of this approach is provided in Sánchez et al., 2019.

In the fifth step of reconstruction, we applied the SLIMEr formalism as previously described for S. cerevisiae. As the acyl chain distribution of R. toruloides is different from S. cerevisiae, for example, the presence of 18:2 and 18:3 acyl chains, this required extensive manual curation of the SLIME reactions, culminating in a lipid-curated draft model with 2,781 reactions, which is less than before curation of lipid metabolism as nonrelevant reactions (based on lipid acyl chain compositions) were discarded. We subsequently populated the model with FAME and lipid class data obtained from mid-exponential phase bioreactor cultivations of R. toruloides on glucose (Tiukova et al., 2019).

### 3.4 | Quality control and validation of rhto-GEM

To transform this functional draft model to the first version of the R. toruloides GEM, additional manual curation was performed wherein, step 6 reactions not connected to the main network, as introduced early in the reconstruction process, were removed. In step 7, remaining template-derived genes were replaced by their R. toruloides orthologs where possible and otherwise deprecated, while in step 8 of the reconstruction the (non-) growth associated maintenance energy was fitted to experimentally determined growth and glucose uptake rates (Shen et al., 2013), and in step 9, the annotation of metabolites and reactions was improved. The resulting model, version 1.2.0, is the first curated genome-scale model of R. toruloides with a total of 2,731 reactions, 2,277 metabolites, and 852 genes (Figure 1b). To track model quality, each time a new model version is released, a memote (Lieven et al., 2018) snapshot report is generated, by running a standardized set of metabolic model tests focusing on for example, annotations and consistency. A full snapshot report of rhto-GEM resulted in a memote test score of 62% (Supporting Information S1). In particular, a low stoichiometric consistency is reported with a memote score of nearly 40%. This is at least partially an artifact of the SLIME reactions whose product stoichiometries are weight-normalized, which allows for direct integration of lipid measurements that are provided as grams per gram dry cell weight.

We structurally compared rhto-GEM with previously reported small-scale models of R. toruloides (Bommareddy et al., 2015; Cañameda et al., 2018) and identified a number of manually curated gene associations and reactions that were differently defined in the semiautomatically reconstructed rhto-GEM. These changes were used to curate the model, to yield version 1.2.1 that was used for further analysis. The maximum theoretical TAG production yields in the small-scale models were slightly lower than the yield predicted from rhto-GEM, which can be attributed to the absence of complex I of the oxidative phosphorylation resulting in lower energy yields (Figure 2a).

To validate rhto-GEM functionality, we compared its growth rate to experimental measured values. From literature, we gathered glucose, glycerol, and xylose uptake rates from R. toruloides bioreactor cultivations...
To evaluate which reactions are stoichiometrically most influential in the oleaginous phenotype of *R. toruloides*, we ran reaction essentiality analysis, where the production of one particular TAG (a major storage lipid) was set as cellular objective and the resulting TAG yield was indicative of the essentiality of each reaction. Comparing oleaginous-essential reactions between three carbon sources (Table 1), the largest differences are related to the carbon assimilation pathway; pentose phosphate pathway reactions are more affecting for lipid production on xylose compared to glycerol. Acetyl-CoA carboxylase, which has previously been identified as an important target for increased TAG production (S. Zhang et al., 2016), was here identified as essential on all tested carbon sources. Many reactions that might be perceived to be essential were not identified as such in our analysis, such as the last step of TAG biosynthesis using fatty acyl-CoA and diacylglycerol as catalyzed by diacylglycerol acyltransferase. However, TAGs can alternatively be generated by reshuffling acyl chains between phospholipids and diacylglycerols. The relatively small number of essential reactions, therefore, demonstrates the high flexibility of lipid metabolism.

3.6 | Prediction of potential targets for increased production of triacylglycerols

Genetic studies of *R. toruloides* have shown that its lipid accumulation capacity can be further enhanced (reviewed in Marella, Holkenbrink, Siewers, & Borodina, 2018). We employed FSEOF on *rhto-GEM* to identify potential metabolic engineering targets for improved production of TAG. FSEOF is based on the principle that increased production requires a redirection of flux, from originally going toward biomass generation, to ideally going (partially) toward our product of interest. However, reactions that already carry significant flux for biomass production are accounted for in FSEOF, as these are potentially less promising targets for overexpression. As there is interest in the use of hydrolyzed plant biomass as feedstock, we evaluated potential targets for both glucose and xylose as carbon source, in addition to the often used glycerol (Table 2 and Figure 3).

Many glycolytic genes were predicted as genetic engineering targets for improved production of TAGs, while enzymes of the pentose phosphate pathway were also identified as targets when cultivated on xylose, as anticipated. All three components of the pyruvate decarboxylase (PDC)-aldehyde dehydrogenase (ALD)-acytela-CoA synthetase (ACS) pathway were predicted to be targets suitable for overexpression under cultivation on xylose, which was unexpected as this pathway is considered less energy efficient than the pyruvate dehydrogenase (PDH)-ATP-citrate lyase (ACL) pathway, which is active in oleaginous yeasts. However, in the non-oleaginous fermentative yeast *S. cerevisiae* upregulation of different members of the PDH bypass was shown to improve TAG accumulation (Shiba, Paradise, Kirby, Ro, & Keasling, 2007), and upregulation of the PDH bypass also improved lipid production in *Y. lipolytica* (Xu, Qiao, Ahn, & Stephanopoulos, 2016).

More of the identified targets have previously been validated by experimental results: that is, overexpression of native acetyl-CoA...
carboxylase (ACC1), diacylglycerol O-acyltransferase (DGA1), glycerol-3-phosphate dehydrogenase (GUT2) genes have all been shown to increase TAG yield in *R. toruloides* (S. Zhang et al., 2016), while increased expression of fatty-acyl-CoA synthase (FAS1 and FAS2) was beneficial in *S. cerevisiae* (Runguphan & Keasling, 2014). Similarly, the prediction that upregulation of stearoyl-CoA desaturase (OLE1) agreed with a published report that overexpression of this enzyme increases lipid production (S. Zhang et al., 2016). This relates to fatty acid composition of TAGs in *R. toruloides*, mostly containing oleic acid at sn-2 position, as prevailing TAGs (~15% each) are POO (16:0, 18:1, 18:1), POP (16:0, 18:1, 16:0), POS (16:0, 18:1, 18:0) and minor TAGs (10%–5% each) are PLO (16:0, 18:2, 18:1), PLP (16:0, 18:2, 16:0), and PLS (16:0, 18:2, 18:0) (Wei et al., 2017). Desaturation of palmitic and stearic acid, which may inhibit acetyl-CoA synthase, has been shown to produce higher-lipid yields in *Y. lipolytica* (Qiao, Wasylenko, Zhou, Xu, & Stephanopoulos, 2017).

Gene targets that have previously been shown to enhance TAG accumulation in *R. toruloides* but were not predicted in the current analysis include nonmetabolic genes such as those involved in organelle morphogenesis, which are currently beyond the capacity of analysis of a purely metabolic model. This include genes such as *RHTO_05627*, which encodes the lipid droplet-associated protein Ldp1 (Zhu et al., 2015) whose expression has been shown to improve lipid production.

### 3.7 Prediction of potential targets for increased production of linolenic acid

Oleaginous yeasts are a potential source of essential fatty acids such as linolenic and linoleic acid, while linolenic ω-3 fatty acids provide health benefits in nutrition and serve as precursors for synthesis of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in humans (Innis, 2014). Natural strains of *R. toruloides* contain linolenic acid around 3% of total fatty acids and the level of fatty acids saturation can change in response to temperature (Suutari, Liukkonen, & Laakso, 1990). Genetic engineering targets for improved production of polyunsaturated fatty acids (PUFAs) in micro-organisms have been reviewed previously (Gong et al., 2014). We performed FSEOF analysis specifically for linolenic acid production, and as expected, most genes identified as targets were also identified for improved production of TAGs, in addition to oleoyl-CoA and linoleoyl-CoA desaturases (Table 2). This is furthermore supported by published reports, for example, overexpression of native Δ9 desaturase (Tsai et al., 2019; S. Zhang et al., 2016) was

### Table 1: Effect on triacylglycerol production after removal of reactions important for oleaginous phenotype

| Glucose | Xylose | Glycerol | Reaction | Reaction name |
|---------|--------|----------|----------|--------------|
| 0       | 0      | 0        | r_0109   | Acetyl-CoA carboxylase, reaction |
| 75      | 65     | 64       | r_0226   | ATP synthase |
| 57      | 24     | 42       | r_0438   | Ferrocytochrome-c:oxygen oxidoreductase |
| 57      | 24     | 42       | r_0439   | Ubiquinol:ferricytochrome c reductase |
| 67      | 60     | 49       | r_1110   | ADP/ATP transporter |
| 95      | 95     | 89       | r_1277   | Water diffusion (cytosol) |
| 0       | 0      | 0        | r_1667   | Bicarbonate formation |
| 63      | 58     | 77       | r_1672   | Carbon dioxide exchange |
| 63      | 58     | 77       | r_1697   | CO2 transport (cytosol) |
| 0       | 100    | 100      | r_1714   | Δ-glucose exchange |
| 100     | 100    | 0        | r_1718   | Δ-xylose exchange |
| 57      | 24     | 42       | r_1978   | O2 transport (mitochondrion) |
| 0       | 0      | 0        | r_1979   | O2 transport (cytosol) |
| 0       | 0      | 0        | r_1992   | Oxygen exchange |
| 58      | 24     | 43       | r_2096   | Water diffusion (mitochondrion) |
| 95      | 95     | 89       | r_2100   | Water exchange |
| 0       | 0      | 0        | r_2183   | Stearoyl-CoA desaturase |
| 0       | 0      | 0        | r_3531   | O2 transport (ER membrane) |
| 0       | 0      | 0        | r_3532   | NADH transport (ER membrane) |
| 0       | 0      | 0        | r_3533   | NAD transport (ER membrane) |

Note: Lipid production as percentage of control strain, after removal of individual reactions, using glucose, xylose, or glycerol as carbon source.
| Triacylglycerol | Linolenic acid | Reaction name | Gene association |
|----------------|---------------|---------------|------------------|
| Glucose | Xylose | Glycerol | Glucose | Xylose | Glycerol | Glucose | Xylose | Glycerol |
| 5 | 20.08 | 20.02 | 20.20 | 6.66 | 6.64 | 6.69 | Acetyl-CoA carboxylase | RHTO_02161 and RHTO_02004 |
| 2 | 16.87 | 16.70 | 5.06 | 5.01 | 6-phosphogluconolactonase | RHTO_07939 |
| 1 | 16.87 | 16.70 | 5.06 | 5.01 | Glucose 6-phosphate dehydrogenase | RHTO_07853 |
| 3 | 16.87 | 16.70 | 5.06 | 5.01 | Phosphogluconate dehydrogenase | RHTO_02788 |
| 4 | 15.05 | 14.45 | 4.86 | 3.60 | Ribulose 5-phosphate 3-epimerase | RHTO_05984 |
| | 22.71 | 0.19 | 0.18 | 7.52 | Pyruvate decarboxylase | RHTO_00098 |
| | 22.71 | 0.19 | 0.18 | 7.52 | Acetaldehyde dehydrogenase | RHTO_001882g or RHTO_02062 or RHTO_04310 or RHTO_04425 or RHTO_05680 or RHTO_05838 |
| | 22.64 | 0.16 | 0.15 | 7.50 | Acetyl-CoA synthetase | RHTO_08027 |
| | 18.77 | 0.75 | 0.72 | 8.13 | Adenylate kinase | RHTO_02749 or RHTO_03117 or RHTO_04701 |
| | 15.00 | 6.84 | Inorganic diphosphatase | RHTO_00177 |
| | 13.97 | 4.08 | Glucose-6-phosphate isomerase | RHTO_04058 |
| 6 | 2.59 | 2.58 | 2.60 | 0.86 | 0.86 | 0.86 | Fatty-acyl-CoA synthase (n-C18:0CoA) | RHTO_02032 or RHTO_02139 |
| | 6.48 | 2.86 | Pyruvate kinase | RHTO_01610 |
| | 6.08 | 2.72 | Enolase | RHTO_00323 |
| | 6.08 | 2.72 | Phosphoglycerate mutase | RHTO_04793 or RHTO_07535 or RHTO_07773 |
| | 4.96 | 2.34 | Glyceraldehyde-3-phosphate dehydrogenase | RHTO_000408g or RHTO_01292 or RHTO_03032 or RHTO_04701 |
| | 4.96 | 2.34 | Phosphoglycerate kinase | RHTO_00033 |
| | 0.63 | 0.63 | 0.64 | 0.87 | 0.87 | 0.88 | Stearoyl-CoA desaturase | RHTO_03911 |
| | 0.87 | 0.87 | 0.94 | 0.94 | Glycerol-3-phosphate dehydrogenase (NAD) | RHTO_00726 or RHTO_01962 |
| 8 | 1.05 | 1.05 | 1.07 | 1-acyl-sn-glycerol-3-phosphate acyltransferase (1-18:0, 2-18:1) | RHTO_05332 or RHTO_06718 |
| | 0.98 | 0.98 | 1.16 | Dihydroxyacetone kinase | RHTO_04669 |
| | 0.98 | 0.98 | 1.16 | Glycerol dehydrogenase (NAD-dependent) | RHTO_00641 or RHTO_03032 or RHTO_03963 or RHTO_06555 or RHTO_07387 |
| 10 | 1.00 | 1.00 | 1.00 | Diacylglycerol acyltransferase (1-18:0, 2-18:1, 3-18:0) | RHTO_00726 or RHTO_01962 |
| 9 | 1.00 | 1.00 | 1.00 | PA phosphatase (1-18:0, 2-18:1) | RHTO_04894 |
| | 0.98 | 0.98 | 1.01 | PA phosphatase (1-16:0, 2-18:3) | RHTO_04894 |
| | 0.98 | 0.98 | 0.99 | DAG lipase (1-16:0, 2-18:3) | RHTO_00361 |
| | 0.98 | 0.98 | 0.99 | Fatty-acid-CoA ligase (linolenate) | RHTO_00058 or RHTO_04350 |
| | 0.98 | 0.98 | 0.98 | Fatty-acid-CoA ligase (palmitate) | RHTO_00058 or RHTO_04350 |
| | 0.98 | 0.98 | 0.98 | 1-acyl-sn-glycerol-3-phosphate acyltransferase (1-16:0, 2-18:3) | RHTO_05332 or RHTO_06718 |
| | 0.98 | 0.98 | 0.98 | MAG lipase (1-16:0:0) | RHTO_03511 |
| 7 | 0.96 | 0.96 | 0.96 | Glycerol-3-phosphate acyltransferase (18:0) | RHTO_03058 |
shown to result in increase of linolenic acid production in \( R. \) toruloides. In the yeast cell, PUFAs may occur as constituent of phospholipids, sulfolipids, acylglycerols, or glycolipids (Jacob, 1992). Fungal \( \Delta 6 \)-desaturases were shown to have preference for acyl groups esterified at the sn-2 position of phosphatidylcholine (PC) over acyl-CoAs. This PC-linked \( \Delta 6 \)-desaturation pathway creates limiting step in synthesis of \( \omega-3 \) fatty acids. Acyl-PCs may be channeled toward TAG synthesis via acyltransferase reaction.

Understanding of substrate specificity of desaturases and acyltransferases could contribute to regulation of PC, acyl-CoAs, TAGs pools, and more accurate determination of fluxes that should be enhanced for improved production of linolenic acid. Overexpression of heterologous desaturases (with desired substrate specificity) from organisms naturally overproducing \( \omega-3 \) fatty acids can be a promising approach as compared to overexpression of native desaturases (Wang, 2013). In \( Y. \) lipolytica overexpression of
heterologous desaturases resulted in enhanced production of ω-3 fatty acids with EPA as final product at the highest content among known EPA sources (Xie, Jackson, & Zhu, 2015; Xue et al., 2013).

3.8 Prediction of potential targets for improved production of carotenoids

Carotenoids are terpenoid pigments that are widely used as natural colorants in food industry (Mata-Gómez, Montañéz, Méndez-Zavala, & Aguilar, 2014). Several genera of basidiomycete yeast are natural producers of carotenoids including Sporobolomyces, Sporidiobolus, Rhodotorula, and Xanthophyllomyces/Phaffia (Bizzini et al., 2007). Many of these yeasts reside in the phylloplane and their ability to produce carotenoids is thought to serve as protection against solar radiation.

Carotenoid production in yeast has been shown to depend on cultivation conditions (Dias, Silva, Freitas, Reis, & da Silva, 2016; Singh et al., 2016) with yields of 0.28 mg/g having been achieved in fed-batch cultivation of R. toruloides (Dias, Sousa, Caldeira, Reis, & Lopes da Silva, 2015). R. toruloides produces a number of carotenoids including torularhodin, torulene, γ-carotene, and β-carotene. Torulene is the major carotenoid, comprising 50% (w/w) of pigment produced, followed by torularhodin and γ-carotene, which account for 20% each. Torulene, which contains 13 double bonds per molecule, has shown to display higher antioxidant activity than β-carotene, which contains 11 double bonds per molecule (Sakaki, Nochide, Komemushi, & Miki, 2002).

We performed FSEOF analysis to predict targets for carotenoid production, with torularhodin as representative product (Table 3 and Figure 3). Most of enzymes within the mevalonate pathway were predicted as targets for overexpression, as this pathway is responsible for the production of the prenyl pyrophosphate precursor. This indicates that a higher flux is required for carotenoids than what is obtained during biomass production. Overexpression of truncated 3-hydroxy-3-methyl-glutaryl-CoA reductase from Kluyveromyces marxianus in combination with other genes was shown to increase β-carotene production in Rhodotorula glutinis (Pi et al., 2018). Similarly, also all enzymes of isoprene biosynthetic pathway were predicted to correlate with improved carotenoid production in R. toruloides. The dimethylallyltranstransferase/geranyltranstransferase (ERG20) is involved in synthesis of farnesy1 pyrophosphate from geranyl pyrophosphate and isopentenyl pyrophosphate, which is a direct metabolic precursor of carotenoids as well as ergosterol, heme A, dolichols, and prenyl-adducts for prenylated proteins. A recent study has demonstrated that overexpression of the X. dendrorhous geranylgeranyl pyrophosphate synthase (encoded by the BTS1 gene), which is immediately downstream of ERG20, increased carotenoid production in R. glutinis (Pi et al., 2018).

Carotenoid biosynthetic enzymes, such as phytoene synthase and dehydrogenase, were identified as targets for increased carotenoid production, as anticipated. The gene coding for phytoene dehydrogenase (RHTO_04602) has been experimentally verified to be involved in carotenoid biosynthesis (Sun et al., 2017). In addition, overexpression of the X. dendrorhous phytoene desaturase and synthase genes was shown to increase carotenoid yields in R. glutinis (Pi et al., 2018).

### Table 3: Comparison of targets predicted from FSEOF for improved torularhodin production on glucose, xylose, or glycerol as carbon source

| Figure 3 | Glucose | Xylose | Glycerol | Reaction name | Gene association |
|----------|--------|--------|---------|---------------|-----------------|
| 12       | 3.96   | 3.95   | 3.96    | Mevalonate kinase (atp) | RHTO_02122 |
| 11       | 3.95   | 3.95   | 3.95    | Hydroxymethylglutaryl CoA reductase | RHTO_04045 |
| 14       | 3.95   | 3.95   | 3.95    | Mevalonate pyrophosphate decarboxylase | RHTO_06005 |
| 13       | 3.95   | 3.95   | 3.95    | Phosphomevalonate kinase | RHTO_02073 |
| 3.51     | 4.11   |        |         | Ribulose 5-phosphate 3-epimerase | RHTO_05984 |
| 1.67     | 1.60   |        |         | 6-phosphogluconolactonase | RHTO_07939 |
| 1.67     | 1.60   |        |         | Glucose 6-phosphate dehydrogenase | RHTO_07853 |
| 1.67     | 1.60   |        |         | Phosphogluconate dehydrogenase | RHTO_02788 |
| 18       | 1.00   | 1.00   | 1.00    | Farnesyltrantransferase | RHTO_01660 or RHTO_02504 |
| 19, 21, 22 | 1.00 | 1.00   | 1.00    | Phytoene synthase | RHTO_04605 |
| 20, 23   | 1.00   | 1.00   | 1.00    | Phytoene dehydrogenase | RHTO_04602 |
| 16       | 0.98   | 0.98   | 0.98    | Dimethylallyltrantransferase | RHTO_01660 |
| 17       | 0.98   | 0.98   | 0.98    | Geranyltrantransferase | RHTO_01660 |
| 15       | 0.98   | 0.98   | 0.98    | Isopentenyl-diphosphate D-isomerase | RHTO_05138 |
|          | 0.04   |        |         | Dihydroxyacetone kinase | RHTO_04669 |
|          | 0.04   |        |         | Glycerol dehydrogenase (NADP-dependent) | RHTO_00641 or RHTO_03032 or RHTO_03963 or RHTO_06555 or RHTO_07387 |

Note: Indicated are slopes derived from FSEOF, indicated of whether gene expression should be increased to direct flux from growth toward production. Only reactions with gene associations are shown. First column refers to numbers in Figure 3.

Abbreviation: FSEOF, flux scanning of enforced objective function.
Little genetic analysis of the endogenous enzymes for carotenoid biosynthesis in \textit{R. toruloides} has been carried out to date. A number of mutants with improved carotenoid yields have been generated but the exact genetic changes responsible have not been reported (Bao et al., 2019; C. Zhang et al., 2016). The earlier mentioned T-DNA mutagenesis study reported decreased carotenoid production upon integration into the intron of \textit{R. toruloides} hypothetical gene \textsc{rtho}00032 or the exon of hypothetical gene \textsc{rtho}07952, which is predicted to encode a bZIP transcription factor (Lin et al., 2017).

The same study also reported that T-DNA insertion into the promoter of hypothetical gene \textsc{rtho}07650 (encoding a putative DUF1479 domain protein) increased carotenoid yields, exemplifying that regulation plays an important role in carotenoid biosynthesis, an assertion that would benefit greatly from integrative analysis of expression data with a comprehensive model of metabolism.

Collectively, the FSEOF results have demonstrated the ability of \textit{rhto-GEM} to provide valuable predictions of targets for improved production of key products in \textit{R. toruloides}, as many of these have experimentally been validated to increase production. This renders \textit{rhto-GEM} as persuasive tool to aid in improving the production of less-studied high-value compounds, in addition as a framework for more detailed analysis of high-producing strains.

### 4 CONCLUSION

Previous studies have presented GEMS of several oleaginous fungal species, including oleaginous ascomycete \textit{Yarrowia lipolytica} (Loira, Duleramo, Nicaud, & Sherman, 2012), zygomycetes \textit{Mortierella alpina} (Ye et al., 2015), and \textit{Mucor circinelloides} (Vengsangnak et al., 2016). Our study presents the first reconstruction of GEM of lipid-accumulating basidiomycete \textit{R. toruloides}, and while we used the S. cerevisiae model as reference for the conserved parts of metabolism, \textit{rhto-GEM} contains unique characteristics including ATP:citrate lyase, which is the main source of acetyl-CoA for lipid synthesis; mitochondrial \(\beta\)-oxidation; a cytoplasmic malic enzyme that provides an alternative to the pentose phosphate pathway for NADPH regeneration; and pathways related to polyunsaturated fatty acids and carotenoid biosynthesis.

The model incorporates knowledge obtained from genomics and proteomics data generated for \textit{R. toruloides} (Zhu et al., 2012) and was validated using cultivation data (Azambuja et al., 2018; Bommardey et al., 2015; Bonturi et al., 2017; Shen et al., 2013), demonstrating good agreement with experimentally reported growth rates. Analysis of the model allowed to identify potential genetic engineering strategies for enhanced lipid production. Some of these genetic targets were found to agree with published experimental studies (Diaz et al., 2018; S. Zhang et al., 2016). As such, \textit{rhto-GEM} emerges as a valuable tool for future analysis of oleaginous and lipid metabolism. An important feature is its distribution through a Git repository, which allows for continuous improvement and tracking of model development.

By providing all relevant scripts to replicate the reconstruction of \textit{rhto-GEM}, we have demonstrated how a new genome-scale model can conveniently be generated, a process greatly aided by RAVEN or alternative solutions such as AutoKEGGRec (Karlsen, Schulz, & Almaas, 2018). Expeditious automated reconstruction of a draft model is followed by manual curation to produce a model accurately representing the in vivo metabolic network. This step remains the most time-consuming involving literature study and comparison of simulations with reported experimental results. For lesser studied organisms, such as many of the oleaginous yeasts, this step has the additional challenge of limited available experimental data. Fortunately, continuous research interest in oleaginous yeasts generates new data and knowledge and this can subsequently be applied to curate and iteratively improve the existing model. As such, a genome-scale model is never finished, it is merely describing the current knowledge. To facilitate this, models should be versioned, and their curation tracked in an accessible development environment, while the quality of each model version should be assured by for example, a dedicated testing suite as memote. Through such an approach emerging knowledge of \textit{R. toruloides} metabolism can easily and reproducibly be used to push \textit{rhto-GEM} as a comprehensive tool, which through its open nature is exemplary suited for involvement of other researchers inside the \textit{R. toruloides} community. A move toward free distribution of model improvements at an early stage will be of strong benefit for the research community, while tracking of changes retains the ability to give credit to the responsible contributors.

### ACKNOWLEDGMENTS

The authors acknowledge Dr. Benjamín José Sánchez (Chalmers University of Technology) for valuable discussions and Dr. Tomas Linder (Swedish University of Agricultural Sciences) for assistance with the figures.

### ORCID

Ievgenia A. Tiukova  http://orcid.org/0000-0002-0408-3515
Eduard J. Kerkhoven  http://orcid.org/0000-0002-3593-5792

### REFERENCES

Andrade, R., Leal, R., Roseiro, J., Reis, A., & da Silva, T. L. (2012). Monitoring \textit{Rhodosporidium toruloides} NCYC 921 batch fermentations growing under carbon and nitrogen limitation by flow cytometry. \textit{World Journal of Microbiology and Biotechnology}, 28(3), 1175–1184. https://doi.org/10.1007/s11274-011-0920-2

Azambuja, S. P. H., Bonturi, N., Miranda, E. A., & Gombert, A. K. (2018). Physiology and lipid accumulation capacity of different \textit{Yarrowia lipolytica} and \textit{Rhodosporidium toruloides} strains on glycerol. \textit{BioRxiv}, 1–18. https://doi.org/10.1101/278523

Bao, R., Gao, N., Lv, J., Ji, C., Liang, H., Li, S., ... Lin, X. (2019). Enhancement of torularhodin production in \textit{Rhodosporidium toruloides} by agrobacterium tumefaciens-mediated transformation and culture condition optimization. \textit{Journal of Agricultural and Food Chemistry}, 67(4), 1156–1164. https://doi.org/10.1021/acs.jafc.8b04667

Bommardey, R. R., Sabra, W., Maheshwari, G., & Zeng, A. -P. (2015). Metabolic network analysis and experimental study of lipid production in \textit{Rhodosporidium toruloides} grown on single and mixed substrates. \textit{Microbial Cell Factories}, 14(1), 36. https://doi.org/10.1186/s12934-015-0217-5
Bonturi, N., Crucello, A., Viana, A. J. C., & Miranda, E. A. (2017). Microbial oil production in sugarcane bagasse hemicellulosic hydrolysate without nutrient supplementation by a Rhodosporidium toruloides adapted strain. Process Biochemistry, 57, 16–25. https://doi.org/10.1016/j.procbio.2017.03.007

Buzzini, P., Innocenti, M., Turchetti, B., Libkind, D., van Broock, M., & Mulinacci, N. (2007). Carotenoid profiles of yeasts belonging to the genera Rhodotorula, Rhodosporidium, Sporobolomyces, and Sporidiobolus. Canadian Journal of Microbiology, 53(8), 1024–1031. https://doi.org/10.1139/W07-068

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Beaver, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. BMC Bioinformatics, 10(1), 421. https://doi.org/10.1186/1471-2105-10-421

Castañeda, M. T., Nuñez, S., Garelli, F., Voget, C., & De Battista, H. (2018). Comprehensive analysis of a metabolic model for lipid production in Rhodosporidium toruloides. Journal of Biotechnology, 280, 11–18. https://doi.org/10.1016/j.jbiotec.2018.05.010

Choi, H. S., Lee, S. Y., Kim, T. Y., & Woo, H. M. (2010). In silico prediction of amino-acid metabolism controls flux to lipid accumulation in Yarrowia lipolytica. NPJ Systems Biology and Applications, 2, 16005. https://doi.org/10.1038/npjbsa.2016.5

Lee, N. K., Cheon, C. J., & Rhee, J. -K. (2018). Anti-obesity effect of red radish coral sprout extract by inhibited triglyceride accumulation in a microbial evaluation system and in high-fat diet-induced obese mice. Journal of Microbiology and Biotechnology, 28(3), 397–400. https://doi.org/10.4014/jmb.1802.02005

Lieven, C., Beber, M. E., Ollivier, B. G., Bergmann, F. T., Chauhan, S., Correa, K., ... Jasper, J. (2018). Memote: A community driven effort towards a standardized genome-scale metabolic model test suite. BioRxiv, 1–26. https://doi.org/10.1101/350991

Lin, X., Gao, N., Liu, S., Zhang, S., Song, S., Ji, C., ... Zhu, B. (2017). Characterization of the carotenoid productions and profiles of three Rhodosporidium toruloides mutants from Agrobacterium tumefaciens-mediated transformation. Yeast, 34(8), 335–342. https://doi.org/10.1002/yea.3236

Loira, N., Dulermo, T., Nicaud, J. -M., & Sherman, D. (2012). A genome-scale metabolic model of the lipid-accumulating yeast Yarrowia lipolytica. BMC Systems Biology, 6(1), 35. https://doi.org/10.1186/1752-0509-6-35

Mata-Gómez, L., Montañez, J., Méndez-Zavala, A., & Aguilar, C. (2014). Biotechnological production of carotenoids by yeasts: An overview. Microbial Cell Factories, 13(1), 12. https://doi.org/10.1186/1475-2859-13-12

Nguyen, L. N., Bormann, J., Le, G. T. T., Stärkel, C., Olsson, S., Nosanchuk, J., ... Schäfer, W. (2011). Autophagy-related lipase FgATG15 of Fusarium graminearum is important for lipid turnover and plant infection. Fungal Genetics and Biology, 48(3), 217–224. https://doi.org/10.1016/j.fgb.2010.11.004

Park, Y. K., Nicaud, J. M., & Ledesma-Amaro, R. (2018). The engineering potential of Rhodosporidium toruloides as a workhorse for biotechnological applications. Trends in Biotechnology, 36(3), 304–317. https://doi.org/10.1016/j.tibtech.2017.10.013

Pil, H. W., Li, W. H., Lin, Y. J., Chang, J. J., Anandharaj, M., & Kao, Y. Y. (2018). Engineering the oleaginous red yeast Rhodotorula glutinis for simultaneous β-carotene and cellulase production. Scientific Reports, 8(1), 2–11. https://doi.org/10.1038/s41598-018-29194-z

Prigent, S., Frioux, C., Dittami, S. M. T., Thiele, S., Lahlimi, A., Collet, G., ... Siegel, A. (2017). Meneco, a topology for degraded genome-wide metabolic networks. PLoS Computational Biology, 13(1), e1005276. https://doi.org/10.1371/journal.pcbi.1005276

Pryszcz, L. P., Huerta-Cepas, J., & Gabaldón, T. (2011). MetaPhors: Orthology and paralogy predictions from multiple phylogenetic evidence using a consistency-based confidence score. Nucleic Acids Research, 39(5), e32. https://doi.org/10.1093/nar/gkq953

Qiao, K., Wasylenko, T. M., Zhou, K., Xu, P., & Stephanopoulos, G. (2017). Lipid production in Yarrowia lipolytica is maximized by engineering cytosolic redox metabolism. Nature Biotechnology, 35(2), 173–177. https://doi.org/10.1038/nbt.3763
Runguphan, W., & Keasling, J. D. (2014). Metabolic engineering of Saccharomyces cerevisiae for production of fatty acid-derived biofuels and chemicals. Metabolic Engineering, 21, 103–113. https://doi.org/10.1016/j.meneng.2013.07.003

Sakaki, H., Nochide, H., Kojimushii, S., & Miki, W. (2002). Effect of active oxygen species on the productivity of torularhodin by Rhodotorula glutinis no. 21. Journal of Bioscience and Bioengineering, 93(3), 338–340. https://doi.org/10.1016/S1389-1723(02)00408-0

Sampaio, J. P. (2011). Rhodospirillum Banno (1967). The Yeasts (3, pp. 1523–1539). https://doi.org/10.1016/B978-0-444-52149-1-00127-0

Shen, H., Gong, Z., Yang, X., Jin, G., Bai, F., & Zhao, Z. K. (2013). Kinetics of continuous cultivation of the oleaginous yeast Rhodospirillum toruloides. Journal of Biotechnology, 168(1), 85–89. https://doi.org/10.1016/j.jbiotec.2013.08.010

Shi, J., Feng, H., Lee, J., & Ning Chen, W. (2013). Comparative proteomics profile of lipid-accumulating oleaginous yeast: An iTRAQ-coupled 2-D LC-MS/MS analysis. PLoS One, 8(12), e85532. https://doi.org/10.1371/journal.pone.0085532

Shiba, Y., Paradise, E. M., Kirby, J., Ro, D. K., & Keasling, J. D. (2007). Gene in Rhodosporidium toruloides formation. Bioresource Technology, 98(13), 2490–2498. https://doi.org/10.1016/j.biortech.2006.09.033

Sun, W., Yang, X., Wang, X., Lin, X., Wang, Y., Zhang, S., Shiba, Y., Paradise, E. M., Kirby, J., Ro, D. K., & Keasling, J. D. (2007). Alternative routes of acetyl CoA synthesis identified by comparative genomic analysis: Involvement in the lipid production of oleaginous yeast and fungi. Microbiology, 153(8), 217–228. https://doi.org/10.1099/mic.0.051946-0

Wang, C., & St. Leger, R. J. (2007). The Metzrhizin anisopliae periplin homolog MPL1 regulates lipid metabolism, apressorial turgor pressure, and virulence. Journal of Biological Chemistry, 282(29), 21110–21115. https://doi.org/10.1074/jbc.M605959200

Xie, D., Jackson, E. N., & Zhu, Q. (2015). Sustainable source of omega-3 eicosapentaenoic acid from metabolically engineered Yarrowia lipolytica: From fundamental research to commercial production. Applied Microbiology and Biotechnology, 99(4), 1599–1610. https://doi.org/10.1007/s00253-014-6318-y

Xu, P., Qiao, K., Ahn, W. S., & Stephanopoulos, G. (2016). Engineering Yarrowia lipolytica as a platform for synthesis of drop-in transportation fuels and oleochemicals. Proceedings of the National Academy of Sciences of the United States of America, 113(39), 10848–10853. https://doi.org/10.1073/pnas.1607295113

Zhang, C., Shen, H., Zhang, X., Yu, X., Wang, H., Xiao, S., ... Zhao, Z. K. (2016). Combined mutagenesis of Rhodospirillum toruloides for improved production of carotenoids and lipids. Biotechnology Letters, 38(10), 1733–1738. https://doi.org/10.1007/s10529-016-2148-6

Zheng, S., Skerker, J. M., Rutter, C. D., Maurer, M. J., Arkin, A. P., & Rao, C. V. (2016). Engineering Rhodospirillum toruloides for increased lipid production. Biotechnology and Bioengineering, 113(5), 1056–1066. https://doi.org/10.1002/bit.25864

Zhu, Z., Zhang, S., Liu, H., Shen, H., Lin, X., Yang, F., ... Zhao, Z. K. (2012). A multi-omic map of the lipid-producing yeast
Rhodosporidium toruloides. Nature Communications, 3, 1112. https://doi.org/10.1038/ncomms2112

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Tiukova IA, Prigent S, Nielsen J, Sandgren M, Kerkhoven EJ. Genome-scale model of Rhodotorula toruloides metabolism. Biotechnology and Bioengineering. 2019; 116:3396–3408. https://doi.org/10.1002/bit.27162