Whole Conversion of Agro-Industrial Wastes Rich in Galactose-Based Carbohydrates into Lipid Using Oleaginous Yeast *Aureobasidium Namibiae*

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Abstract

Background

Raw materials composed of easily assimilated monosaccharides have been employed as carbon source for production of microbial lipids. Nevertheless, agro-industrial wastes rich in galactose-based carbohydrates have not been introduced as feedstocks for oleaginous yeasts.

Results

In this study, *Aureobasidium namibiae* A12 was found to efficiently accumulate lipid from soy molasses and whey powder containing galactose-based carbohydrates, with lipid productions of 5.30 g/L and 5.23 g/L, respectively. Over 80% of the fatty acids was $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$. All kinds of single sugar components in the two byproducts were readily converted into lipids, with yields ranging between 0.116 g/g and 0.138 g/g. Three α-galactosidases and five β-galactosidases in the strain were cloned and analyzed. β-galactosidase was responsible for lactose hydrolysis; sucrase and α-galactosidase both contributed to the efficient hydrolysis of raffinose and stachyose in a cooperation manner.

Conclusions

This is a new way to produce lipids from raw materials containing galactose-based carbohydrates. This finding revealed the significance of sucrase in the direct hydrolysis of galactose-based carbohydrates in raw materials for the first time and facilitated the understanding of the efficient utilization of galactose-based carbohydrates to manufacture lipid or other chemicals in bioprocess

Background

Microbial lipids can be produced with high efficiency and no competition from edible oil and have become important sources for producing biodiesel and other chemicals now (Ma et al., 2018). Among the lipid producing microorganisms, oleaginous yeasts are attractive lipid producers for their rapid growth, large biomass, and high lipid yield (Wang et al., 2013; Munch et al., 2015). The reported oleaginous yeasts mainly belong to several genera, such as *Aureobasidium, Candida*, *Cryptococcus*, *Lipomyces*, *Rhodotorula*, *Rhodosporidium*, *Trichosporon*, and *Yarrowia* (Cho et al., 2018). Among them, *Lipomyces starkeyi*, *Yarrowia lipolytica*, and *Rhodosporidium toruloides* can accumulate high level of neutral lipids, over 60% of their dry cell weight, from different feedstocks (Wang et al., 2018; Cho et al., 2018; Takaku et al., 2020).

At present, the high production cost, especially the cost of carbon source for fermentation, impedes the industrial applications of microbial lipids (Cho et al., 2018). In view of this, accumulating lipid from low-cost substrates is a feasible strategy to lower the production cost. A variety of raw materials or bio-wastes including corncob, corn stover, wheat straw, cassava starch, waste oils, inulin, sugarcane bagasse,

and sugarcane molasses, have been employed as carbon source for the lipid production by oleaginous yeasts (Cho et al., 2018).

Overall, the carbohydrates in these raw materials above mainly rely on glucose or fructose as monosaccharide unit, which can be easily assimilated. However, some agro-industrial by-products contain plenty of galactose-based carbohydrates, such as lactose and raffinose-family oligosaccharides (RFO) (Wang et al., 2019). The galactoside bonds in the sugars are difficult to break up, thereby leading to limited applications and lowered commercial value. Due to lacking galactosidase synthesis and intracellular galactose assimilation pathway, most oleaginous yeasts cannot naturally consume galactose and RFO (Wang et al., 2019). In addition, construction of galactose metabolic pathways in most yeast was easy to achieve (Mano et al., 2020). The abundant agro-industrial wastes containing galactose-based carbohydrates, such as whey powder (WP) and soy molasses (SM), have not been introduced as carbon source for lipid production (Wang et al., 2019; Mano et al., 2020).

SM is a main by-product of the soy protein extraction from soybean. As soybean is one kind of staple crop providing plant proteins and lipid, SM is produced in large amount. Generally, SM has a high carbohydrate content of above 30% (w/v), mainly containing stachyose, raffinose, sucrose, monosaccharides, and other micronutrients (Dong et al., 2014; Romão et al., 2012; Siqueira et al., 2008). Owing to the existence of α-(1,6) glycosidic bonds in RFO, SM cannot even be efficiently utilized by non-ruminant animals (Wang et al., 2019). WP is a by-product in cheese production with an annual worldwide production of about 165 million tons (Macwan et al., 2016). Despite whey containing many fermentable nutrients, such as lactose, proteins, and lipids (Blazeck et al., 2014), its utilization is estimated to be only 75% in Europe and less than 50% in the rest of the world (Macwan et al., 2016).

Aureobasidium has recently been evaluated as an excellent lipid producer and reported to efficiently assimilate various monosaccharides (Wang et al., 2018). This genera was found to secrete many kinds of hydrolytic enzymes involved in biomass degradation (Wang et al., 2018). In our previous study, several galactosidases were detected among extracellular substances of *Aureobasidium* sp., indicating the ability of assimilating galactose-based carbohydrates (Zhang et al., 2019). In this study, *Aureobasidium namibiae* strain A12 derived from mangrove was screened to accumulate high content of lipid directly from WP and SM.

Results

Evaluation of lipid producers of the yeast strains from SM

Apart from raffinose and stachyose, SM contained high level of conventional sugars, such as glucose, fructose, and sucrose, accounting for more than 40% of total carbohydrates (Wang et al., 2019). These conventional sugars provided enough carbon sources for lipid accumulation of *L. starkey* and *R. toruloides*, with the lipid production of 2.42 g/L and 2.51 g/L, respectively (Fig. 1). The highest lipid content (47.3%, w/w) was observed in the strain A12 system, with the lipid production of 5.30 g/L and the

biomass of 11.20 g/L (Fig. 1). Obviously, only the conventional sugars in SM cannot support the large-scale lipid synthesis of strain A12. This indicated that the RFO should be converted into lipid in strain A12. The low content of residual sugar also provided another evidence for this.

Evaluation of lipid producers of the yeast strains from WP

Many researchers have made efforts to convert WP into citric acid, lactic acid, and lipases (Yalcin et al., 2009; Silva et al., 2014; Arslan et al., 2016). WP also has received a wide attention to serve as the raw material for the production of biofuels recently (Mano et al., 2020). *A. namibiae* strain A12 and other four typical oleaginous yeasts were evaluated from the perspective of the capability to produce lipid from WP, whose main component is lactose. As shown in Fig. 2, strain A12 possessed the highest lipid content (48.6%, w/w), able to produce 5.23 g/L lipid and 10.72 g/L biomass from WP. On the contrary, *L. starkeyi*, *Y. lipolytica*, and *R. toruloides* can only accumulate a small amount of biomass and lipid compared with strain A12. This indicated that strain A12 can utilize lactose as the main component in WP, while the other three oleaginous strains only adopt the monosaccharides (such as glucose and fructose) with low concentrations as carbon source.

Composition of the fatty acids

Triacylglycerols (TAGs), the reported main components of lipid in oleaginous yeasts, were stored in intracellular lipid droplets. There existed 1–3 droplets in each cell of strain A12. After being stained by Nile Red, the lipid droplets presented yellow fluorescence which can be observed under a fluorescent microscope with blue excitation light (Athenstaedt, 2010). GC analysis of the fatty acids showed that the content of linoleic acid ($C_{18:2}$), oleic acid ($C_{18:1}$), stearic acid ($C_{18:0}$), palmitoleic acid ($C_{16:1}$), and palmitic acid ($C_{16:0}$) was 9.83%, 57.42%, 10.51%, 2.1%, and 20.14%, respectively. The composition of the fatty acids was rather similar to that of plant oils (Cho et al., 2018). As revealed by previous experiments, the long-chain fatty acids constituted by 16 and 18 carbon atoms were ideal candidates for biodiesel production via methylation.

Analysis on the utilization of different sugars

To further demonstrate the ability of strain A12 to utilize the waste sugars with galactoside bonds, it was cultured in a lipid-producing medium in which a single component in SM and WP was employed as the sole carbon source, including glucose, galactose, fructose, lactose, sucrose, xylose, stachyose, and raffinose. As shown in Fig. 3, the lipid production, biomass, and residual sugars in these fermentations have been compared. The test monosaccharides, which are present in SM and WP or exist as monosaccharide units of other sugars, were all readily converted to lipid. The lipid yield from them was not much different, ranging from 0.116 g/g to 0.138 g/g. This suggests that an efficient galactose and metabolism pathway indeed exists in the strain A12. It should be noted that this strain can even convert xylose into lipid, which is quite challenging for many other yeasts (Cho et al., 2018). Besides, it can utilize the sucrose, commonly used as a conventional carbon source, and galactose-based sugars in an efficient manner, revealing the existence of a developed galactosidase system (Carota et al., 2017; Vyas et al., 2019).

Galactosidase system in strain A12

α-Galactosidase is effective in catalyzing the generation of α-linked galactose residues from various substrates, thus being crucial for the conversion of RFO into conventional carbon source sucrose; while β-galactosidase is able to cut the β-linked galactose residue of lactose to yield glucose (Wang et al., 2019; Mano et al., 2020). Based on the genome sequence of the type strain for this species, *A. namibiae* CBS 147.97, the potential galactosidase coding gene in strain A12 was cloned and analyzed. As listed in Table 1, diverse α-galactosidases and β-galactosidases were constituted by different numbers of amino acids. Through analysis, three potential α-galactosidases (namely GalA, GalB, and GalC) and five potential β-galactosidases (i.e., GalD, GalE, GalF, GalG, and GalH) were found. α-Galactosidases in strain A12 have been classified into two glycoside hydrolase families GH27 and GH36. GH27 and GH36 are perceived to have a common ancestral gene and hence they share a common catalytic mechanism. The phylogenic tree showed that GalB and GalC from GH27 are clustered into the same branch, while GalA belongs to another branch. Each α-galactosidase of *A. namibiae* A12 is found very close to that of *Aspergillus niger*. This is in accordance with the recognition that the genus *Aureobasidium* is close to *A. niger* in terms of evolutionary relationship (Prasongsuk et al., 2018). Nevertheless, β-galactosidases of *A. namibiae* A12 from two glycoside hydrolase families are much more diverse. Although the five β-galactosidases in *A. namibiae* A12 are all separated from β-galactosidases from plants and bacteria, they diverge in four branches close to specific basidiomycetes or ascomycetes, forming subclasses of specialized enzymes.

Table 1
Potential α-galactosidases and β-galactosidases in strain A12 and the basic characteristics.

Proteins	Accession	Function	Super family	Amino acids	Signal peptide
GalA	MW302897	α-galactosidase	GH36	750	Yes
GalB	MW302898	α-galactosidase	GH27	452	Yes
GalC	MW302899	α-galactosidase	GH27	522	No
GalD	MW298673	β-galactosidase	GH2	858	No
GalE	MW298674	β-galactosidase	GH35	1001	Yes
GalF	MW298675	β-galactosidase	GH35	1002	Yes
GalG	MW298676	β-galactosidase	GH35	1009	Yes
GalH	MW298673	β-galactosidase	GH7	1071	No

Coordination of glycoside hydrolases during the lipid production

The hydrolysis of carbohydrates in the medium can be realized by inducing microorganisms to generate corresponding glycoside hydrolases. To elucidate the role of glycoside hydrolases in the medium

containing SM or WP as the carbon source, their activities and the concentrations of different sugars were monitored at a 12 h interval in a 10 L fermentor. The different activities of these glycoside hydrolases can be attributed to the different concentrations of the corresponding enzyme inducers, the substrates of the enzymes in the medium. β-galactosidase activity was 7.6 U/mL at 24 h in WP medium, while the activities of α-galactosidase and sucrase were at low levels of 0.6 U/mL and 1.3 U/mL, respectively. Galactose and glucose derived from lactose hydrolysis were then directed to lipid and biomass synthesis. After 72 h, no lactose was detected and the β-galactosidase started to decrease. At 96 h, galactose was completely consumed, with a maximal lipid production of 6.13 g/L. Adequate β-galactosidase enables the utilization of lactose in WP medium.

α-Galactosidase is responsible for converting raffinose-family oligosaccharides into conventional sucrose and galactose. As Fig. 5a showed, α-galactosidases and sucrase were induced much more than those in WP, with 22.6 g/L RFO detected. At 24 h, the activity of α-galactosidase reached 6.52 U/mL, with reduced content of stachyose and increased galactose; the activity of sucrase reached 4.60 U/mL. However, the content of raffinose just decreased slightly to 3.51 g/L at 36 h, as a result of the supplement through the release of raffinose from stachyose cut by α-galactosidase. Besides, the content of sucrose even increased to the level before 24 h. This indicated that the hydrolysis of sucrose by sucrase was slower than its generation from stachyose and raffinose catalyzed by α-galactosidase. At 48 h, the activity of α-galactosidase and sucrase reached 13.2 U/mL and 7.2 U/mL, respectively. Under the reaction of α-galactosidase and sucrase, sucrose and RFO were both not detected after 96 h, and the lipid achieved a maximal yield of 6.45 g/L.

Discussion

As far as we know, this is the first time for the introduction of SM as low-cost carbon source in lipid production (Cho et al., 2018; Dong et al., 2014; Romão et al., 2012; Siqueira et al., 2008). Benefiting from the low cost and easy availability, SM has become a promising feedstock in China (Wang et al., 2019). Moreover, the high carbohydrate content makes SM attractive among researchers and biorefinery industrialists (Cho et al., 2018; Dong et al., 2014; Romão et al., 2012; Siqueira et al., 2008). However, the current SM-derived fermentations suffer from limited efficiency, as most of them still focused on the utilization of the conventional constituents (Cheng et al., 2016; Dong et al., 2014; Solaiman et al., 2004; Yang et al., 2018). Up to now, only the synthesis of poly-hydroxyalkanoate, propionic acid, and polymalic acid from RFO in SM was achieved (Cheng et al., 2016; Full et al., 2006; Yang et al., 2018).

The tested typical oleaginous yeasts in this study all have been proved capable of accumulating high level of lipid from various carbohydrates with glucose or fructose as the monosaccharide unit. *R. toruloides* accumulates lipid from cassava starch (Park et al., 2018). Inulin, sucrose, and tuber meal of Jerusalem artichoke have been used for lipid production in *Aureobasidium pullulan* (Wang et al., 2018). The lipid content and lipid production of *Y. lipolytica* Po1g grown in sugarcane bagasse hydrolysate were 58.5% and 6.68 g/L, respectively (Wang et al., 2020). Moreover, *R. toruloides*, *L. starkeyi*, and *Y. lipolytica* can produce lipid with the hydrolysate derived from the pretreatment of wheat straw (Yu et al., 2011). As

for galactose-based carbohydrates, only *A. namibiae* among the oleaginous yeasts can convert them into lipid.

Generally, very few yeasts possess β-galactosidase and galactose metabolism pathway, thus resulting in the impossibility to utilize lactose by most yeasts (Domingues et al. 2010). In previous studies, only two oleaginous yeasts have been reported able to accumulate lipid from WP with a content of more than 40% (Carota et al., 2017; Vyas et al., 2019). *Cystobasidium oligophagum* has been characterized to efficiently convert WP into lipid. The biomass and the lipid content were 43.1% and 6.38 g/L, respectively (Vyas et al., 2019). In another study, the biomass and lipid production of *Cryptococcus curvatus* from WP achieved 10.77 g/L and 63.4%, respectively (Carota et al., 2017). Moreover, a secreted β-galactosidase was introduced in *Y. lipolytica*, along with enhanced the cellular galactose metabolism. The engineered strain could achieve the rapid conversion of acid whey, producing 6.61 g/L of fatty acids (Mano et al., 2020). *A. namibiae* was proved to also be an efficient lipid producer from WP for the first time.

Generally, similar compositions were found in the composition of fatty acids in the majority of oleaginous yeasts, including *Y. lipolytica*, *L. starkeyi*, and strain A12 (Cho et al., 2018; Takaku et al., 2020). These results confirmed that SM and WP would be the ideal substrates for lipid production. Although the fermentations in SM and WP were probably slowed down due to the hydrolysis of raffinose and stachyose, lower osmotic pressures were allowed and the biomass was increased. Thus, as largely untapped resources currently, SM and WP deserve arousing attention from *A. namibiae* system to be a self-sufficient nutrient for numerous potential bioprocesses (Cao et al., 2010). This galactosidases system provides molecular basis for strain A12 to utilize sugars with galactoside bonds. And the galactosidase in *Aureobasidium* can be a valuable untapped industrial enzyme resource (Prasongsuk et al., 2018).

Unexpectedly, two kinds of sugars not present in SM medium have been detected during the fermentation, which were identified as melibiose and galacto-oligosaccharide. Their existence revealed the neglected fact that the sucrase also functions in the hydrolysis of stachyose and raffinose apart from α-galactosidase (Trindade et al., 2003). Sucrase has been proved to catalyze the release of fructose residues from different substrates including stachyose and raffinose. Thus, sucrase and α-galactosidase both contribute to efficient hydrolysis of RFO in a cooperation manner, rather than in a sequential way (Cao et al., 2010; Trindade et al., 2003). The sequence of cutting the two kinds of glycosidic bonds influences the generation of intermediate sugars, as shown in Fig. 6. In the case of sole α-galactosidase, RFO was converted into sucrose with the release of galactose; when only sucrose exists, melibiose and galacto-oligosaccharide were generated with the release of fructose (Fig. 6). This finding revealed the significance of sucrase in the direct hydrolysis of RFO in raw materials for the first time and facilitated the understanding of the efficient utilization of RFO to manufacture lipid or other chemicals in bioprocess (Wang et al., 2019; Cho et al., 2018).

Conclusions

A. namibiae A12 efficiently accumulate lipid from galactose-based SM and WP. All kinds of single sugar components in SM and WP were readily converted into lipid, producing similar amount of lipid. β-galactosidase was responsible for lactose hydrolysis in WP; sucrase and α-galactosidase both contributed to the efficient hydrolysis of RFO in a cooperation manner. This is a new way to produce lipid from galactose-based raw materials. This platform strain can be further improved to produce high-value lipid derived chemicals from waste sugars with galactoside bonds.

Methods

Strains and media

Four typical oleaginous yeast strains, i.e., *R. toruloides* R2, *Trichosporon fermentans* T1, *Y. lipolytica* URA, and *L. starkeyi* AM were preserved at − 80 ℃ in our laboratory. Strain A12 was isolated from a plant in Zhanjiang Mangrove National Nature Reserve, and identified as *A. namibiae*. Yeast strains were cultivated in YPD medium (containing 20.0 g/L glucose, 20.0 g/L peptone, and 10.0 g/L yeast extract) (Madzak, 2015). The composition of lipid production (LP) medium included 50 g/L glucose, 3.0 g/L K_2HPO_4, 2.0 g/L KH_2PO_4, 0.1 g/L $MgSO_4{\cdot}7H_2O$, and 2 g/L corn steep liquor (CSL), with pH 6.0. When other pure carbohydrates were selected as the carbon source, the concentrations of the carbohydrates remained 50 g/L. In the experiments checking the lipid production from the wastes, SM and low protein WP concentrations were set at 130 g/L and 60 g/L, respectively so that the sugar content in the medium can be maintained at about 50 g/L.

Pretreatment of SM

SM was adjusted to pH 10.0 with Ca $(OH)_2$ powder which was held for 1 h. Subsequently, the liquid was filtered to remove excess Ca $(OH)_2$ and adjusted to pH 6.0 with H_2SO_4, which was kept for 1 h. After 10 min of boiling, the solution was centrifuged at 7000×*g*, followed by being stored at 4°C (Zhang et al., 2019). The pretreated SM contained 3.32% (*w*/*w*) glucose, 2.81% (*w*/*w*) fructose, 13.42% (*w*/*w*) sucrose, 1.73% (*w*/*w*) xylose, 3.15% (*w*/*w*) raffinose, 14.26% (*w*/*w*) stachyose, and 0.78% (*w*/*w*) galactose.

Lipid production at flask level

Strain A12 was cultivated in 5 mL YPD medium at 30 ℃ for 20 h which was then switched to 50 mL LP medium at the same temperature. The fermentation proceeded for a total of 120 h, after which the broth was centrifuged at 5000×*g*. The yeast cells were harvested by centrifugation at 5000×*g*, washed with sterile water, and dried at 80 ℃ overnight for the biomass detection. Nile Red (0.5 mg/L in DMSO, GenMed Scientifics Inc., USA) was adopted to stain the washed cells (10 μL) which were then observed under an Olympus U-LH100HG fluorescent microscope with blue excitation light (Wang et al., 2018).

Molecular manipulations

The extraction of genomic DNA from the *A. namibiae* strain was conducted with the TIANamp Yeast DNA Kit (TIANGEN BIOTECH, China). The DNA polymerase was utilized according to the recommendation by

the manufacturer (New England BioLabs, USA). The transformation of *E. coli* was realized by the heat shock method of Sambrook et al. (1989). The primers were designed based on the potential gene encoding galactosidase in *A. namibiae* CBS 147.97, and this potential gene in strain A12 was subjected to PCR amplification. The amplified DNA fragments were then transformed into *E. coli* DH5α (TaKaRa Biotechnology, China). The *E. coli* transformants were screened on LB agar which contained 100.0 μg/mL ampicillin and play a role in the plasmid amplification and DNA sequencing.

Bioinformatics analysis of galactosidases

The gene protein sequence was compared using HMMER3 based on the CAZy (Carbohydrate-Active enZYmes) database. In this way, the annotation information of carbohydrate-active enzymes became available (Cantarel et al., 2018). E-value < 1e-5 was adopted as the filter condition. NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/) was used for the prediction of N-glycosylation sites, and the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP-4.1/) was applied to the signal peptide analysis. The phylogenetic tree was constructed on the basis of reported α-galactosidase and β-galactosidase sequences by virtue of the neighbor-joining method in MEGA version 7.0.

Determination of fatty acid composition of the extracted oil

The cellular lipids were extracted and weighed with the method of Folch et al. (1957). For the sake of determining fatty acid composition, the oil obtained by Soxhlet extraction was directly subjected to transmethylation and fatty acid esters were then analyzed using gas chromatography (GC) according to the literature procedure (Li et al., 2010).

Enzymatic activity assay and sugar concentration determination

The α-galactosidase activity was evaluated with pNPG as substrate (Cao et al., 2010). The reaction was terminated by Na_2CO_3 and the solution was filtered through a 0.22 μm membrane, followed by high-performance liquid chromatography (HPLC) analysis. The carbohydrate concentration was calculated depending on the retention time and peak area. One unit of the α-galactosidase activity (U) was defined as the amount of the enzyme that generated 1 μM *p*-nitrophenol per minute at 40°C in buffer at pH 4.5.

The detection of β-galactosidase activity was carried out by the similar method (Juers et al., 2010). The mixture of the culture (0.5 mL) and *o*-nitrophenyl-β-D-galactopyranoside (ONPG, 20.0 mM, 0.5 mL) in 100 mM citrate buffer (pH 4.0) underwent the incubation at 40°C for 10 min. The β-galactosidase in the mixture was inactivated by 2.0 mL of 0.5 M Na_2CO_3 solution. One unit of the β-galactosidase activity (U) was defined as the amount of the enzyme producing 1.0 μM ONP per minute in the conditions of this study.

The sucrase activity was detected as reported (Zhang et.al, 2016). The carbohydrate content was determined on an Agilent 1200 system (Agilent Technologies, USA) with amino (NH_2) column (Thermo Scientific, USA).

Lipid production in 10 L fermentor

Large-scale fermentation was performed in Biostat B 10 Lfermentors (B. Braun, Germany) for the lipid production from WP and SM respectively. Strain A12 was first inoculated in YPD medium (600 mL) as a seed culture and then transferred to the fermentor which contained 6 L of lipid production medium. In the next step, the fermentation began and lasted for 120 h under the following conditions: agitation speed: 300 rpm, aeration rate: 50 L/min, temperature: 30 ℃, and pH 6.0. The samples were also taken every 12 h to determine the biomass, lipid yield, and activities of α-galactosidase, β-galactosidase, and sucrase. Besides, the sugar content was monitored as well.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent for publications.

Competing interests

The authors declare that they have no competing interest.

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Figures

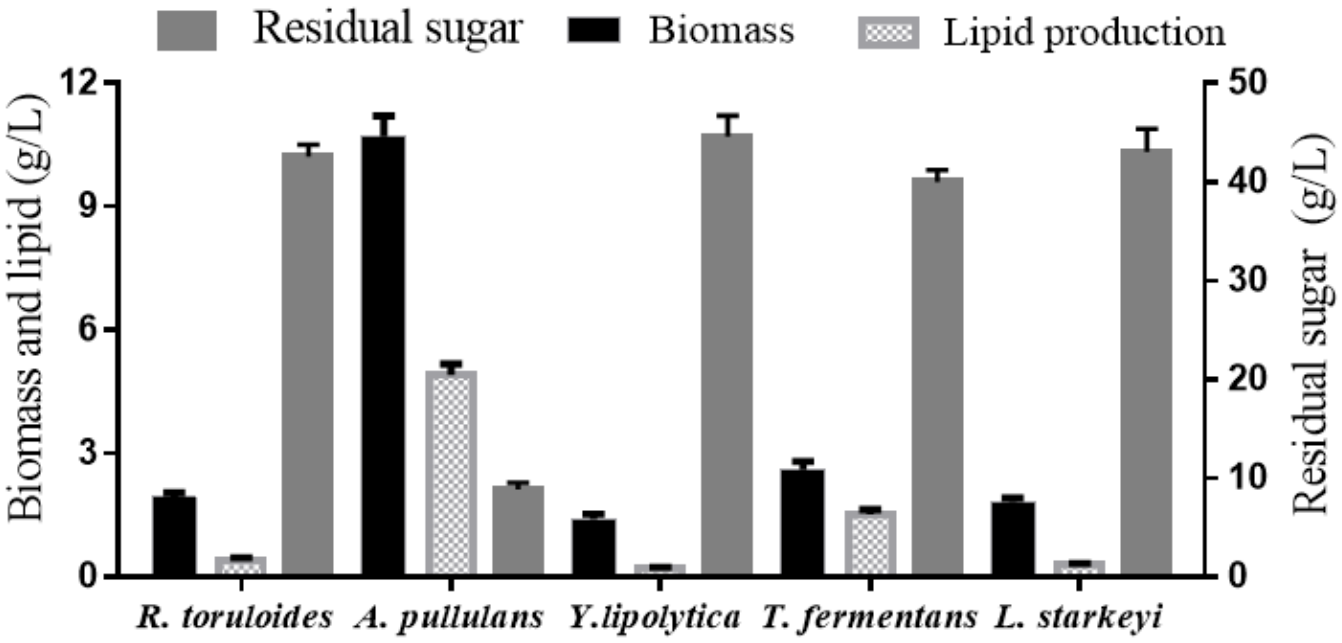

Figure 1

Lipid productions of typical oleaginous yeasts and strain A12 from SM. Data are given as means ± standard deviation, n = 3.

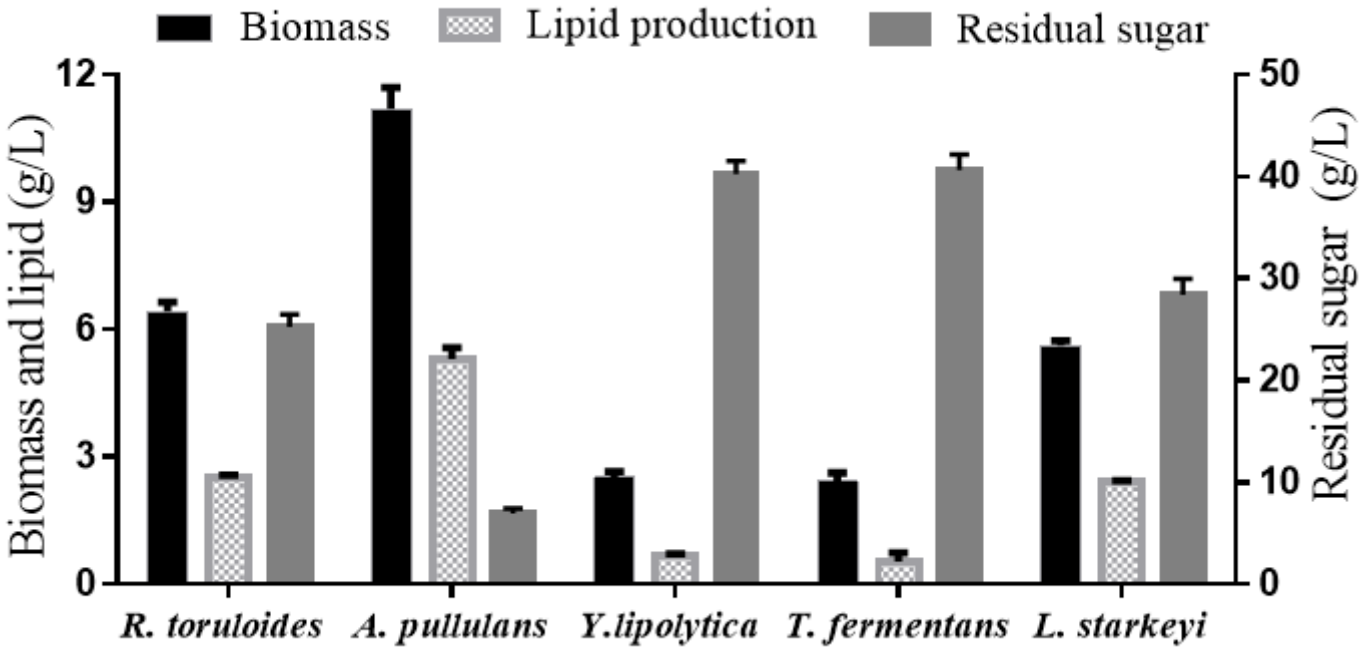

Figure 2

Lipid productions of typical oleaginous yeasts and strain A12 from WP. Data are given as means ± standard deviation, n = 3.

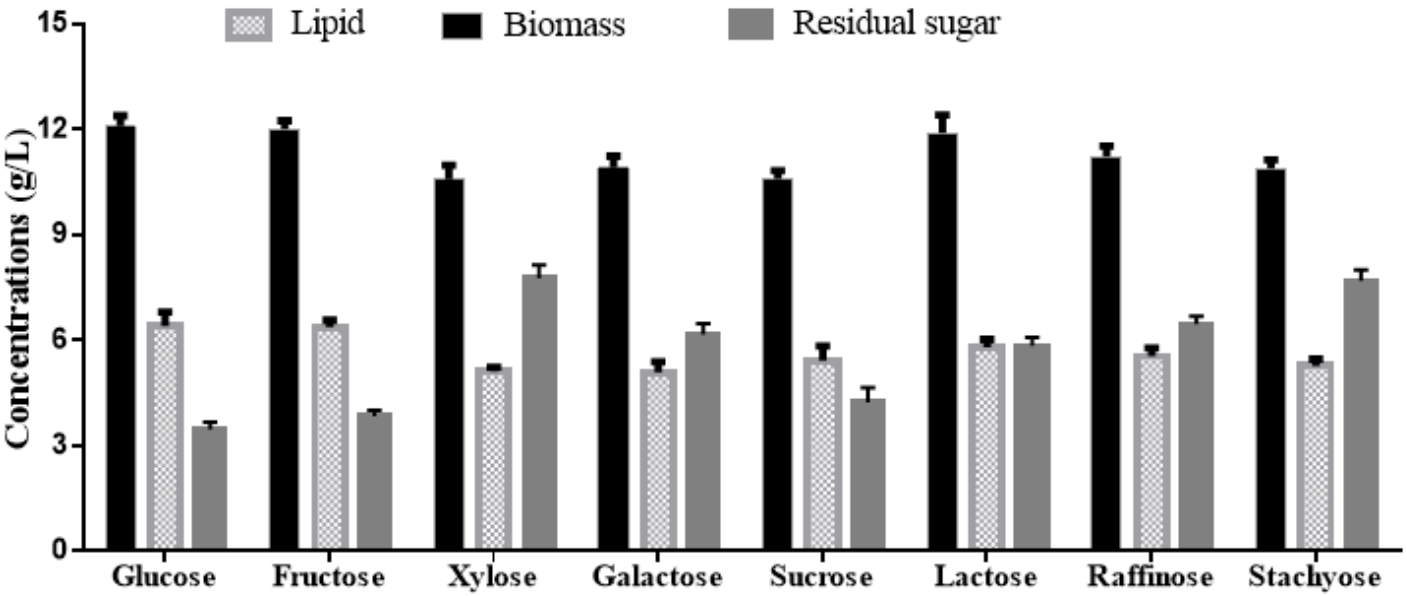

Figure 3

Lipid productions of strain A12 from single sugar components in SM and WP. Data are given as means ± standard deviation, n = 3.

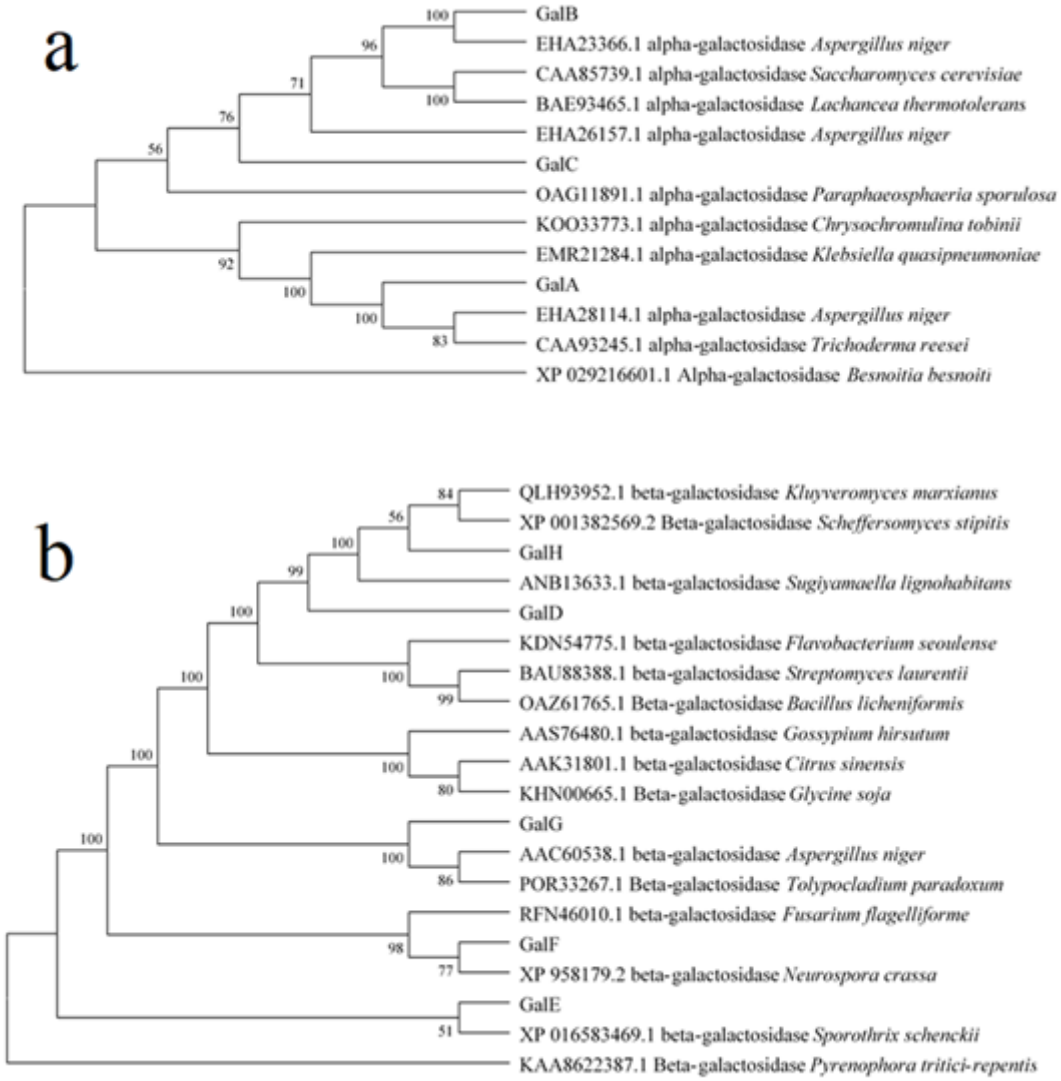

Figure 4

The phylogenetic tree generated with the neighbor-joining method based on α-galactosidase (a) and β-galactosidase sequences (b). Branch-related numbers are bootstrap values (confidence limits).

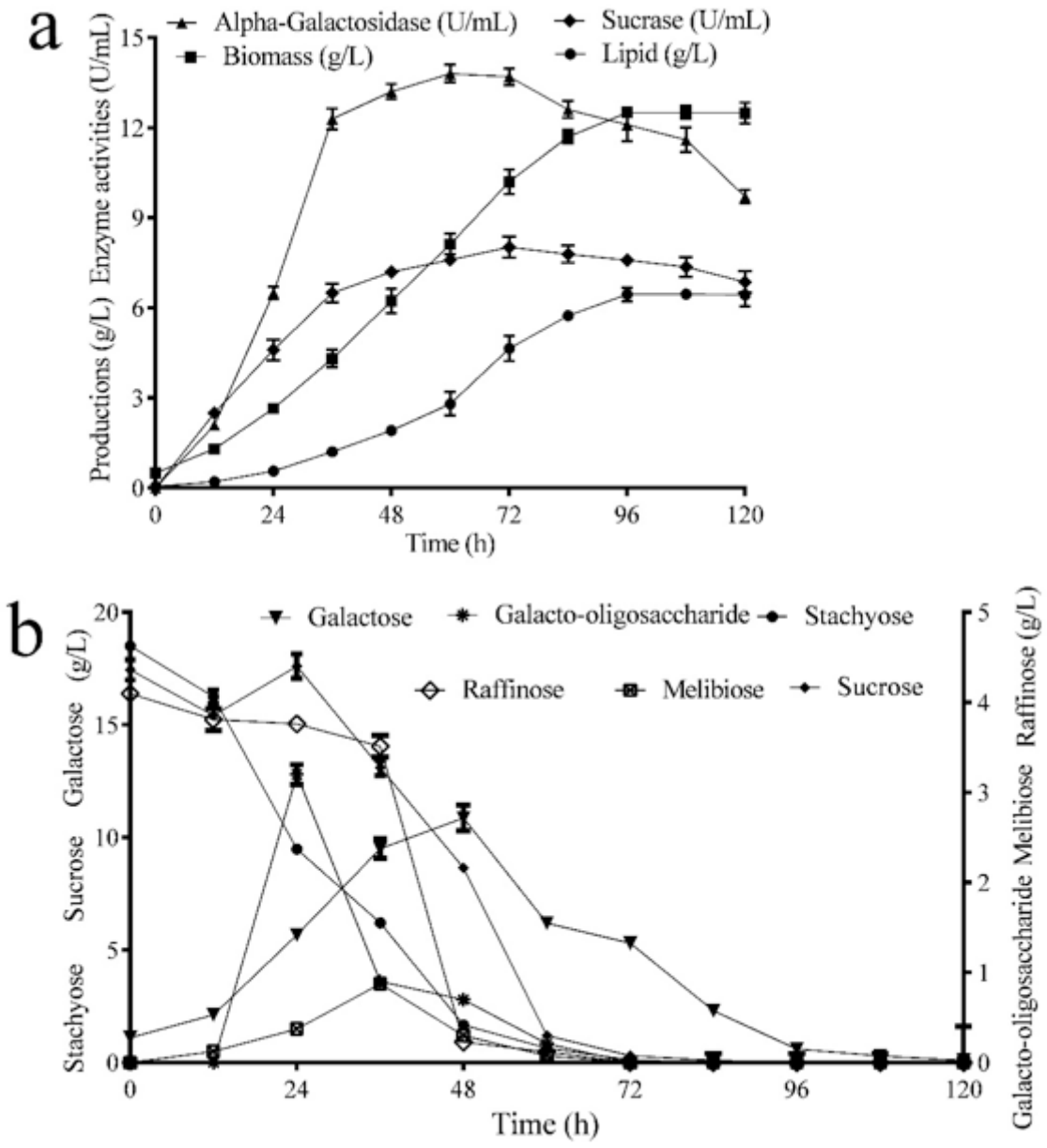

Figure 5

Time course of lipid, biomass, sucrase and α-galactosidase in the 10-L bioreactor during fermentation by strain A12 (a). Time course of sugar contents during fermentation by strain A12 (b). Data are given as means ± standard deviation, n = 3.

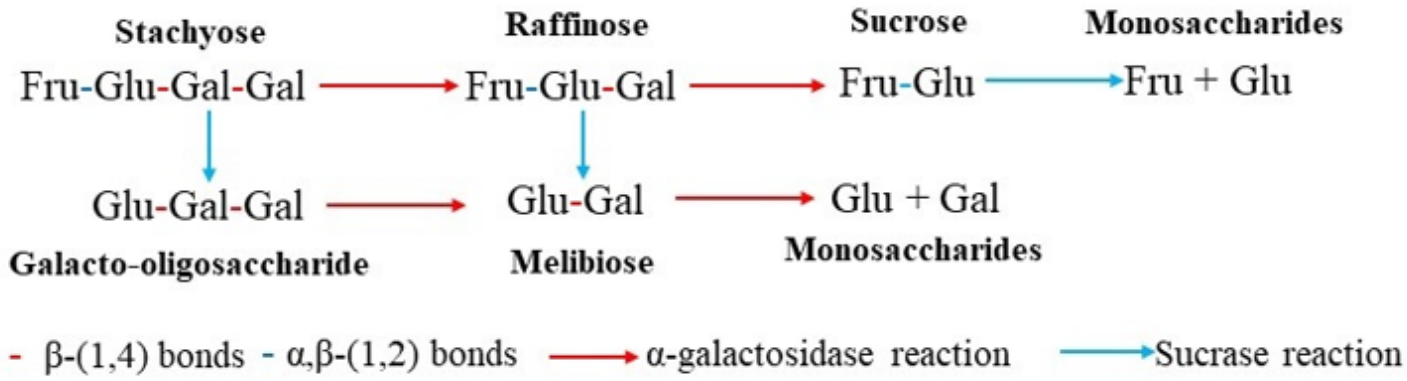

Figure 6

The contribution of sucrase and α-galactosidase to the hydrolysis of RFO. Glu: Glucose; Fuc: Fructose; Gal: Galactose

Supplementary Files

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