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# Characterization of Inulin Hydrolyzing Enzyme(s) in Oleaginous Yeast *Trichosporon cutaneum* in Consolidated Bioprocessing of Microbial Lipid Fermentation

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**Abstract** Oleaginous yeast *Trichosporon cutaneum* CGMCC 2.1374 was found to utilize inulin directly for microbial lipid fermentation without a hydrolysis step. The potential inulinase-like enzyme(s) in *T. cutaneum* CGMCC 2.1374 were characterized and compared with other inulinase enzymes produced by varied yeast strains. The consolidated bioprocessing (CBP) for lipid accumulated using inulin was optimized with 4.79 g/L of lipid produced from 50 g/L inulin with the lipid content of 33.6 % in dry cells. The molecular weight of the enzyme was measured which was close to invertase in *Saccharomyces cerevisiae*. The study provided information for inulin hydrolyzing enzyme(s) in oleaginous yeasts, as well as a preliminary CBP process for lipid production from inulin feedstock.

**Keywords** Inulin hydrolyzing enzyme · *Trichosporon cutaneum* · Dry cell weight · Lipid production · Consolidated bioprocessing

## Introduction

Microbial lipid has a similar composition with plant oil and is considered as an alternative feedstock for biodiesel production [1]. Typical oleaginous bacteria, fungi, yeasts, and algae produce over 30 % (*w*/*w*) intracellular lipid of their cell mass [2]. Among these microbes, oleaginous yeasts such as *Yarrowia lipolytica, Rhodosporidium toruloides*, *Lipomyces starkeyi*, *Trichosporon fermentans*, *Rhodotorula mucilaginosa*, *Trichosporon capitatum*,

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*Apiotrichum curvatum*, *Candida curvata,* and *Cryptococcus curvatus* are advantageous for their fast growth rate, high oil content, and the resemblance of their triacylglycerol fraction to plant oil [3, 4]. When polysaccharides such as starch and lignocellulose are used as feedstock, a hydrolysis step is required to convert polysaccharides into monosaccharides for lipid fermentation. Recently, a consolidated bioprocessing (CBP) concept is proposed by combining hydrolyzing enzyme production, hydrolysis, and fermentation in a single step for the purpose of cost reduction [5]. Generally, oleaginous microbes are not considered to be able to synthesize hydrolyzing enzyme(s) and secrete it out of the cells. Therefore, a separate hydrolysis step is required using corresponding amylase or cellulase enzymes. However, the CBP concept is often effective applied when inulin is used as the feedstock for lipid fermentation.

Inulin is a fructose oligomer linked linear β-(2–1) bond from Jerusalem artichoke, dahlia tubers, or other plant. Studies showed that many hydrolase enzymes have similar properties of inulin hydrolysis into fructose and glucose, such as inulinase [6], β-glucosidase [7], invertase [8], and glucoamylase [9]. The hydrolyzed fructose and glucose can be fermented into ethanol [10], lactic acid [11], or lipid [3, 12] by oleaginous yeasts. Yeast strains such as *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Pichia guilliermondii* are capable of inulin hydrolysis [13, 14]. Wang and Li [15] confirmed that invertase SUC2 is the key hydrolase for converting inulin into fructose and glucose in *S. cerevisiae*. Wang et al. [16] reported an oleaginous yeast *R. toruloides* 2F5 with inulinase activity and lipid accumulation property, shaping a preliminary CBP example. It is still not yet known whether this is the only inulin-hydrolyzing oleaginous yeast, or a common property among oleaginous yeasts. Also, a clear characterization of inulin hydrolyzing enzyme(s) such as synthesis, secretion, and lipid accumulation of CBP strain is required in the further practical applications.

In this study, the CBP property of an oleaginous yeast *Trichosporon cutaneum* CGMCC 2.1374 was evaluated for lipid accumulation using inulin as feedstock. The properties of inulin hydrolyzing enzyme(s) in *T. cutaneum* CGMCC 2.1374 were assayed. The present study provided a basis for CBP strain development using inulin feedstock.

## Materials and Methods

### Raw Material

Inulin powder was purchased from Tianhe New Hisense Co., Guangzhou, Guangdong, China. For determination of inulin composition, the complete hydrolysis was carried out in 4 M HCl solution at 120 °C for 30 min. The hydrolysate was filtrated through the 0.22-μm filter and analyzed on HPLC. Inulin contained 58.83 % of fructosan, 8.98 % of glucan, as well as 10.32 % of glucose and 5.83 % of fructose by weight percentage.

Yeast extract was purchased from Oxoid, Basingstoke, Hampshire, UK. Dithiothreitol (DTT) was purchased from Fermentas, Shenzhen, China. Methanol, fructose, and chloroform were purchased from Sinopharm Chemical Reagent Co., Shanghai, China. 3,5-Dinitrosalicylic acid, peptone, and ethylenediaminetetraacetic acid ($Na_2EDTA$) were purchased from Shanghai Kefeng Industrial Co., Shanghai Chemical Reagent Chemical Supply Station Center, and Bio SHARP, respectively. All other chemicals including glucose, phenol, sodium potassium tartrate tetrahydrate, $Na_3PO_4$, $KH_2PO_4$, $(NH_4)_2SO_4$, $MgSO_4$, HCl, $H_2SO_4$, $Na_2SO_3$, phenol, boron trifluoride, sodium citrate, and citrate were of reagent grade and purchased from the local supplier Lingfeng Chemical Reagent Co. in Shanghai, China.

## Strains and Media

*T. cutaneum* CGMCC 2.1374, *Rhodotorula glutinis* CGMCC 2.703, *R. toruloides* CGMCC 2.1609, and *K. marxianus* var. *marxianus* CGMCC 2.1549 were purchased from the China General Microbiological Culture Collection Center, Beijing, China (http://www.cgmcc.net/). *S. cerevisiae* DQ1 was a mutant strain obtained in our previous study [17] and stored at the Chinese General Microorganisms Collection Center, Beijing, China, with the registration number of CGMCC 2528. *Y. lipolytica* DSM3286 was purchased from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (http://www.dsmz.de/home.html).

These yeast strains were cultured in yeast peptone dextrose (YPD) medium (20 g/L of glucose, 20 g/L of peptone and 10 g/L of yeast extract) at 30 °C, 180 rpm for 12 h, then 10 % of the culture was inoculated into fresh YPD medium at 30 °C, 180 rpm for 24 h as the seed culture.

The fermentation medium used contained 10–90 g/L of inulin, 0.5 g/L of yeast extract, 0.22 g/L of $(NH_4)_2SO_4$, 0.5 g/L of $MgSO_4{\cdot}7H_2O$, and 1.0 g/L of $KH_2PO_4$.

The inulinase fermenting medium contained 20 g/L of inulin, 1.0 g/L of yeast extract, 1.0 g/L of $(NH_4)_2SO_4$, 2.0 g/L of $KH_2PO_4$, and 1.0 g/L of $MgSO_4{\cdot}7H_2O$.

## Lipid Fermentation

*T. cutaneum* CGMCC 2.1374 was cultured in YPD medium at 30 °C, 180 rpm for 12 h for activation and then 10 % of the culture was inoculated into fresh YPD medium at 30 °C, 180 rpm for 24 h for seed cultivation; lastly, 10 % of the seed culture inoculated into the fermentation medium for lipid fermentation. The culture was carried out in a 500-mL conical flask containing 50 mL medium at 30 °C for 120 h with the initial pH of 5.0. pH in the flask was constant during the culture of *T. cutaneum* CGMCC 2.1374 because of no acidic or alkaline intermediate generation. The aeration in the flask is sufficient for cell growth and lipid accumulation. All experiments were performed twice and the error ranges were given.

For lipid extraction, 30 mL of fermentation broth was taken and centrifuged at 10,000*g* for 5 min to harvest the yeast cells, then washed and dried at 80 °C for 24 h until a constant weight. The cell biomass was determined gravimetrically. The lipid was extracted using the chloroform-methanol method according to Folch et al. [18]. The extracted lipid was directly transmethylated according to the procedure [19]. The fatty acid compositions of the total lipid samples were analyzed as their methyl ester by gas chromatograph-mass spectrometer (GC-MS). The GC was a Clarus 500, PerkinElmer, with an HP-5 column of 30 m × 0.25 mm × 0.25 μm in size and helium gas at 1 mL/min; the initial oven temperature of 80 °C for 3 min and then with the increase gradient of 16 °C per minute, to the final of 280 °C, and the MS was a Clarus 500, PerkinElmer, with electron impact at 70 eV, charge-mass ratio range at 33–500.

## Activity Assay of Inulin Hydrolyzing Enzyme(s)

The cell samples were centrifuged at 10,000*g* at 4 °C for 10 min, then washed with 0.1 M pH 5.0 citrate buffer twice. Glass beads were added and vigorous vortex mixing was performed for 4 min, then centrifuged at 10,000*g* at 4 °C for 10 min. The obtained crude enzyme supernatant was used for the enzyme activity assay using the method by Jing et al. [20]. Briefly, 0.1 mL of the crude enzyme solution was added to 0.9 mL of citrate buffer solution (0.1 M, pH 5.0) containing 2 % (*w*/*v*) inulin powder and incubated at 50 °C for 30 min. An equal amount of

enzyme solution inactivated by boiling at 100 °C for 10 min was used as control. The reaction was terminated at 100 °C for 10 min. The liberation of reducing sugars was estimated by the dinitrosalicylic acid (DNS) method, using fructose as the standard. One unit (U) of inulin hydrolyzing enzyme was defined as the amount of enzymes that produced 1 μmol of reducing sugars per minute from inulin under the assay conditions.

The protein concentration was measured by the Bradford method, using bovine serum albumin (BSA) as standard. The specific activity was calculated as units per milligram crude protein.

The fraction of extracellular inulin hydrolyzing enzymes was determined by measuring the units in the supernatant of fermentation broth. The distribution of inulin hydrolyzing enzyme(s) in intracellular and periplasmic fractions was estimated using the method in Westphal et al. [21]. Briefly, the cell pellets were resuspended in the extraction buffer at pH 7.0 (containing 50 mM sodium phosphate, 10 mM DTT, and 1 mM $Na_2EDTA$) to extract the periplasm fraction inulin hydrolyzing enzyme. The resuspended cell pellets were permeabilized by adding 80 μL/mL of chloroform and 40 μL/mL 10.0 % SDS and vigorous vortex mixing for 10 s, and the intracellular inulin hydrolyzing enzyme activity of intracellular and periplasmic fractions was determined.

### Purification of Inulin Hydrolyzing Enzyme(s)

The crude inulinase solution was filtered through a 0.45-μm membrane and purified using a chromatography column packed with Sephadex G-100 gel (GE Healthcare, Sweden). The column was pre-equilibrated and eluted with citrate buffer (0.1 M, pH 5.0) at a flow rate of 1.0 mL/min, and the protein concentrations as measured by A280nm readings of 2.0 ml samples from the fraction collector and the volume activity (U/mL) of the crude enzyme fractions. The inulin hydrolyzing enzyme was lyophilized for storage. The fractions of lyophilized inulin hydrolyzing enzyme were dissolved in a small volume of distilled water for SDS-PAGE electrophoresis with 10 % polyacrylamide denaturing SDS gel. The proteins were stained with Coomassie brilliant blue R-250.

### Determination of Sugars in the Fermented Media

Glucose and fructose were analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column at 65 °C. The mobile phase was 5 mM $H_2SO_4$ at the flow rate of 0.6 mL/min. All samples were centrifuged to remove the cells and other water-insoluble solids and filtered through a 0.22-μm filter before analysis.

For inulin, all samples were adjusted to pH 2.0 with 3.0 M chlorhydric acid, heated for 30 min at 120 °C, and filtrated through a 0.22-μm filter; the glucose and fructose released were determined by HPLC. The sum of glucose and fructose was equal to inulin content.

## Results and Discussion

### Production of Inulin Hydrolyzing Enzyme(s) by *T. cutaneum* CGMCC 2.1374

The lipid accumulation in *T. cutaneum* CGMCC 2.1374 cells was measured when different sugars were used as substrates, including glucose, fructose, sucrose, and

inulin (Fig. 1). The dry cell weight (DCW) was 9.29 g/L and lipid concentration was 2.45 g/L when inulin was used without inulinase enzyme addition. The result indicates that like *R. toruloides* [16], *T. cutaneum* CGMCC 2.1374 has similar properties of production and secretion of inulin hydrolyzing enzyme(s) for inulin hydrolysis and then accumulated lipid intracellular.

The activity of inulin hydrolyzing enzyme(s) in *T. cutaneum* CGMCC 2.1374 increased during the first 8 h then kept constant (Fig. 2). The maximum volumetric activity was 0.0719 U/mL at 12 h and the maximum specific activity was 0.1960 U/mg (crude protein) at 28 h. The highest volumetric activity was observed at 25 °C, and the maximum specific enzyme was at 40 °C (Fig. 3a); pH decreased to 2.0–3.0 within 24 h, no matter the difference of the initial pH (3.0–6.5) (Fig. 3b). Organic nitrogen sources, yeast extract, peptone, and beef extract facilitated the cell growth and activity of inulin hydrolyzing enzyme(s) than inorganic nitrogen sources $(NH_4)_2SO_4$ and $NaNO_3$ (Fig. 3c). Glucose, fructose, and sucrose facilitated the cell growth, but lactose, starch, and inulin facilitated inulin hydrolyzing enzyme(s) activity (Fig. 3d). Specific activity decreased with increasing inulin concentration and the maximum activity appeared at 20 g/L of inulin (Fig. 3e).

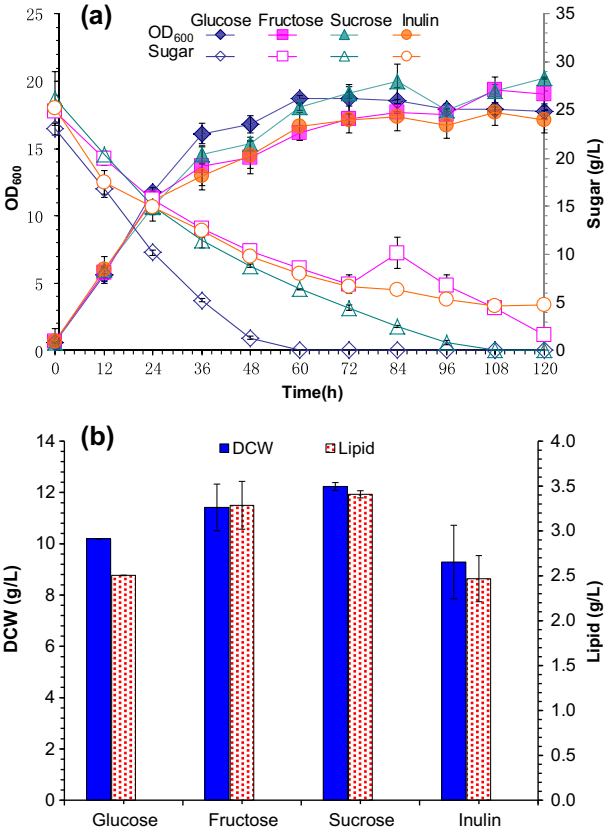



**Fig. 1** Utilization of varied sugars *T. cutaneum* CGMCC 2.1374 for microbial lipid production. **a** the cell growth ($OD_{600}$) and sugar consumption curve; **b** the microbial lipid production. Culture conditions—pH at 5.0, 30 °C for 120 h at 180 rpm

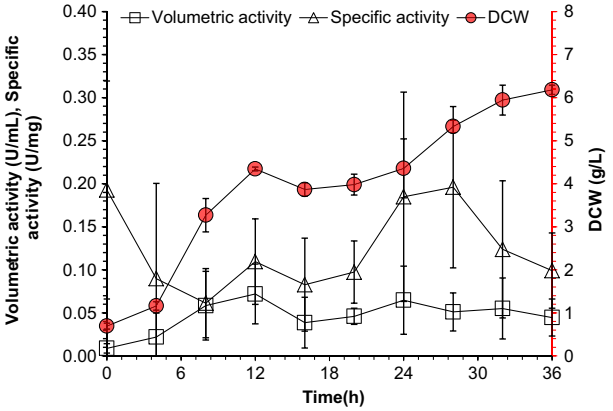



**Fig. 2** Inulin hydrolysis enzyme(s) activity in *T. cutaneum* CGMCC 2.1374. Strain was inoculated in the fermenting medium at pH at 5.0, 30 °C

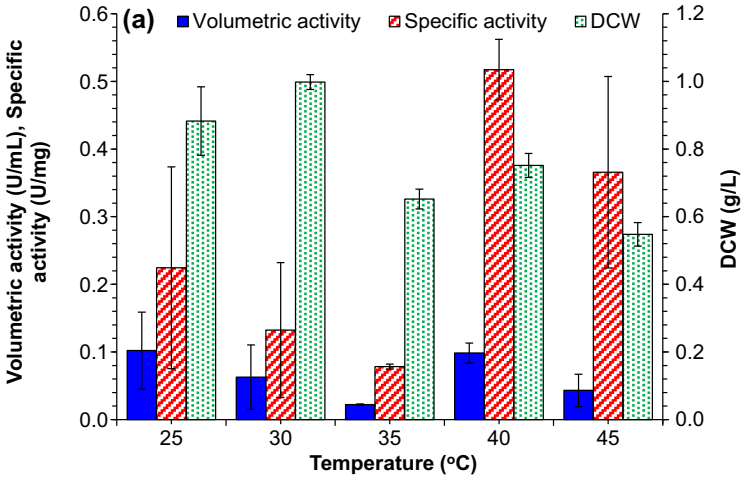
## Characterization of Inulin Hydrolyzing Enzyme(s) in *T. cutaneum* CGMCC 2.1374

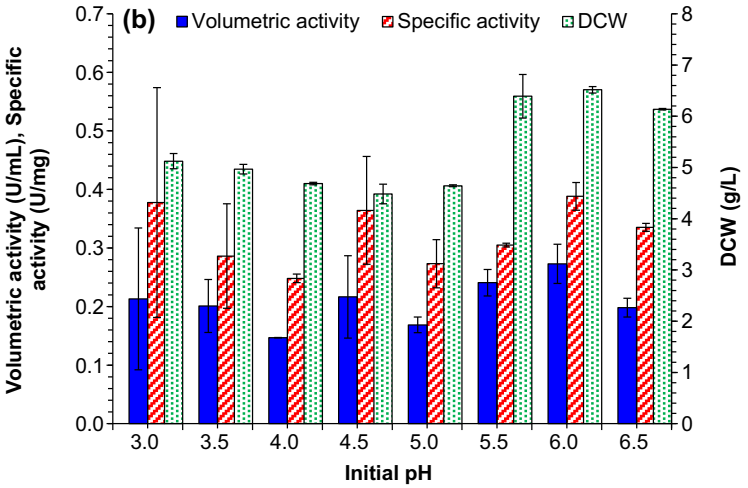
The properties of inulin hydrolyzing enzyme(s) in *T. cutaneum* CGMCC 2.1374 were characterized using crude enzyme solution extracted from cells. Figure 4 shows that 56.45 % of the total inulin hydrolyzing enzyme(s) in *T. cutaneum* CGMCC 2.1374 was in the periplasm and supernatant, indicating the majority of inulin hydrolyzing enzyme(s) was able to secrete into locations where inulin was able to reach. Comparing to only 43 % of the total inulin hydrolyzing enzyme(s) in the periplasm and negligible in supernatant of *S. cerevisiae* DQ1 [22], *T. cutaneum* CGMCC 2.1374 showed better secretive ratio of inulinase-like enzyme.

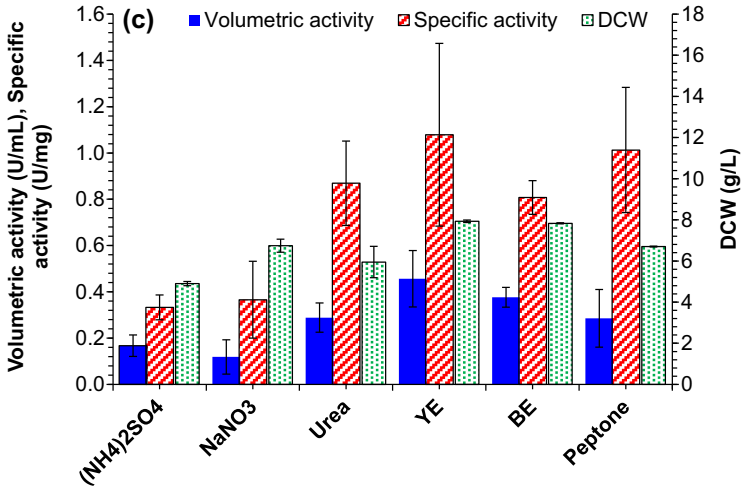
Optimal temperature and pH for inulin hydrolyzing enzyme(s) production in *T. cutaneum* CGMCC 2.1374 were 55 °C and pH 5, respectively (Fig. 5). The volumetric activity increased with increasing temperature from 30 to 55 °C and reached the maximum of 0.121 U/mL at 55 °C, then declined with further increase in temperature. Similarly, the activity was approximately stable in the pH range of 3.5 to 4.5, then increased with further pH increase and reached the maximum at 5.5, and sharply reduced after pH was greater than 5.5. The thermostability was assayed in the range of 30–70 °C for 1 h and the remaining activity was measured. The stability declined with increasing temperature and the optimal was at 30 °C in the range of 30–70 °C. The stability of inulin hydrolyzing enzyme was assayed at 20 °C for 24 h in the pH range of 3.5 to 6.5. The most suitable pH range was 4.0–6.0, and the activity decreased quickly beyond this range.

The inulinase activity of seven selected yeast strains were compared (Fig. 6). Among three ethanol production yeast, the volumetric activity and specific activity of *K. marxianus* var. *marxianus* CGMCC 2.1549 were the highest one (15.053 U/mL, 26.212 U/mg), followed by *S. cerevisiae* DQ1 (12.677 U/mL, 13.123 U/mg) and *Pichia stipitis* (0.305 U/mL, 1.094 U/mg); furthermore, based on the excellent inulin

**Fig. 3** Effect of fermentation parameters on inulin hydrolysis enzyme(s) activity of *T. cutaneum* CGMCC 2.1374. **a** Temperature; **b** initial pH; **c** nitrogen sources; **d** carbon sources; **e** inulin concentration. Culture conditions—initial pH at 5.0, 30 °C, 180 rpm for 24 h


(a)
Volumetric activity
Specific activity
DCW
Volumetric activity (U/mL), Specific activity (U/mg)
DCW (g/L)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.0
0.2
0.4
0.6
0.8
1.0
1.2
25
30
35
40
45
Temperature (°C)


(b)
Volumetric activity
Specific activity
DCW
Volumetric activity (U/mL), Specific activity (U/mg)
DCW (g/L)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
1
2
3
4
5
6
7
8
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
Initial pH


(c)
Volumetric activity
Specific activity
DCW
Volumetric activity (U/mL), Specific activity (U/mg)
DCW (g/L)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
2
4
6
8
10
12
14
16
18
(NH4)2SO4
NaNO3
Urea
YE
BE
Peptone

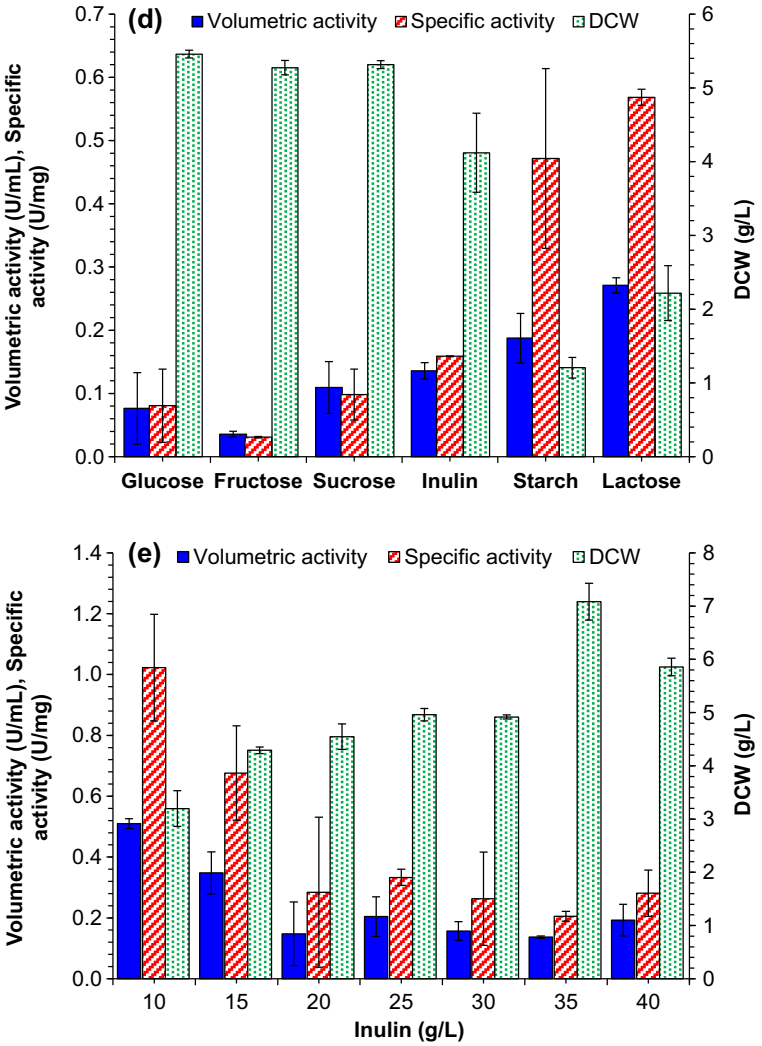



**Fig. 3** (continued)

consumption capability, the CBP bioethanol production technology from Jerusalem artichoke by *K. marxianus* [23] and *S. cerevisiae* [22] has been achieved already. Among four oleaginous yeasts tested, *R. toruloides* 2.1609 showed the highest volumetric activity and specific activity (1.037 U/mL, 17.126 U/mg), followed by *T. cutaneum* CGMCC 2.1374 (0.179 U/mL, 0.227 U/mg); the value of the other two oleaginous yeasts were obviously lower than that, especially *Y. lipolytica* (0.008 U/mL, 0.033 U/mg). All the activity value of the oleaginous yeasts was lower than bioethanol-produced ones, especially *K. marxianus* showed significantly higher volume activity. The volumetric activity or specific activity of *T. cutaneum* CGMCC 2.1374 was about 84 or 115 times lower than those of *K. marxianus*. Thus, we could get the conclusion that inulin hydrolyzation was one of the limiting steps in the CSP

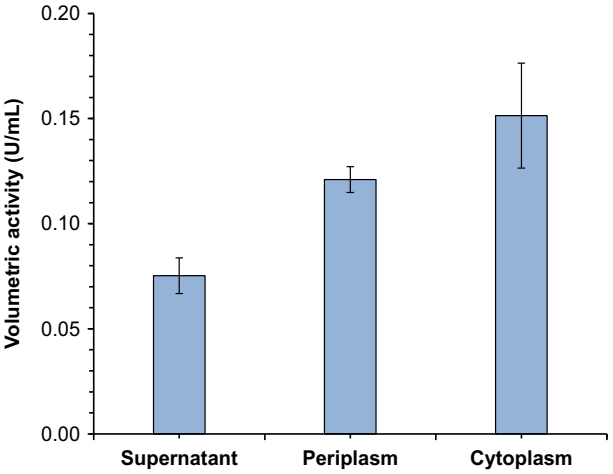



**Fig. 4** Subcellular distribution of inulinase activity in *T. cutaneum* CGMCC 2.174. Culture conditions—initial pH at 5.0, 30 °C, 180 rpm, for 24 h

of *T. cutaneum* CGMCC 2.1374. So improving the inulin hydrolyzing enzyme activity of *T. cutaneum* CGMCC 2.1374 would contribute to the cell growth and lipid accumulation further more.

The crude enzyme solution from *T. cutaneum* CGMCC 2.1374 was collected and purified using Sephadex G-100 column according to the procedure described in the "Materials and Method" section for inulin hydrolysis enzyme(s) activity assay. The elution profile (Fig. 7a) showed three major protein peaks but only the third one exhibited inulin hydrolyzing activity. The SDS-PAGE showed the single protein band had the molecular weight around 80 kDa (Fig. 7b). Inulin hydrolysis could be catalyzed by various β-fructofuranosidase promiscuous enzymes of glycoside hydrolase family [24], such as inulinase (EC 3.2.1.80; EC 3.2.1.7) [25], levansucrase (EC 2.4.1.10) [26], levanase (EC 3.2.1.65) [27], and invertase (EC 3.2.1.26) [28]. On the BRENDA database (http://www.brenda-enzymes.org/index.php), there are totally 15 kinds of inulin hydrolyzing enzymes from three classes: hexosyltransferases (EC 2.4.1.-), glycosidases (EC 3.2.1.-), and acting on polysaccharides enzymes (4.2.2.-), among these enzymes endoinulinase (EC 3.2.1.7), exoinulinase (EC 3.2.1.80), and beta-fructofuranosidase (EC 3.2.1.26) are responsible for the inulin degradation in yeast. In *K. marxianus*, it was endoinulinase or exoinulinase in charge of this reaction with the molecular weight of 77.0 kDa [29] or 62.2 kDa [30], while the molecular weight of exoinulinase function in *Meyerozyma guilliermondii* and *R. toruloides* were 57.8 kDa [31] and 50.5 kDa [16], respectively. The typical inulin hydrolyzing enzyme in yeast is invertase (EC 3.2.1.26) with different molecular weights of 60.7 kDa in *S. cerevisiae* JZ1C [15] and 75.8 kDa in *S. cerevisiae* S288C [32]. The endoinulinase in *Y. lipolytica* was estimated to be 62.4 kDa based on its nuclear sequence [33]. The molecular mass of the purified enzyme in this study seems to be like the molecular weight with invertase in *S. cerevisiae* S288C. Most of inulin hydrolyzing enzymes is protein-secreted; glycosylation is critical for the secreting process [34], for example some invertase of *S. cerevisiae* was the glycosylated protein. However, it was

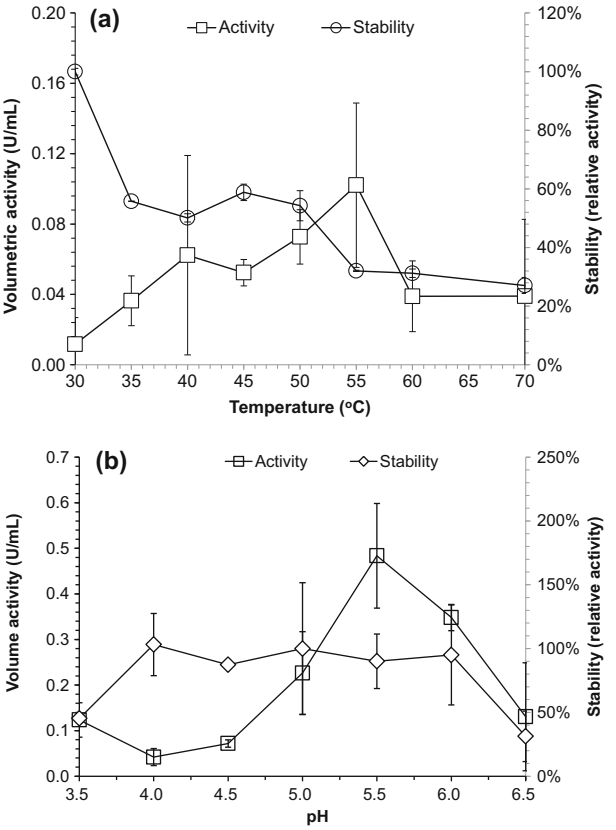



**Fig. 5** Effect of reaction parameters on inulin hydrolysis enzyme(s) activity in *T. cutaneum* CGMCC 2.1374. **a** Temperature, determined at pH 5.0 for 30 min, and stability was determined at pH 5.0 for 1 h. **b** Initial pH, determined at 50 °C for 30 min, and stability was determined at 20 °C for 24 h

uncertain whether the inulin hydrolyzing enzyme of *T. cutaneum* CGMCC 2.1374 was glycosylated protein or not, which needs cloning and expression of its gene to confirm this, for its low expression resulting into unachievable of the purified enzyme deglycosylation.

## Optimizing Lipid Accumulation in *T. cutaneum* CGMCC 2.1374 Using Inulin Feedstock

The optimal lipid accumulation condition of *T. cutaneum* CGMCC 2.1374 using inulin feedstock was searched in a practical experimental range as shown in Fig. 8. Urea was found to be the best nitrogen source for cell growth and lipid accumulation with the dry cell weight and lipid production of 11.06 and 2.62 g/L, respectively, followed by peptone, $(NH_4)_2SO_4$, and $NH_4Cl$ (Fig. 8a). The effect of initial pH on lipid accumulation is not significant (Fig. 8b). Relatively, the highest lipid titer of 2.75 g/L was obtained at the initial pH 5.0, while the highest dry cell weight of 11.32 g/L was obtained at pH 6.0. The cell growth and lipid accumulation increased with inulin concentration significantly in the range of 10–90 g/L, then

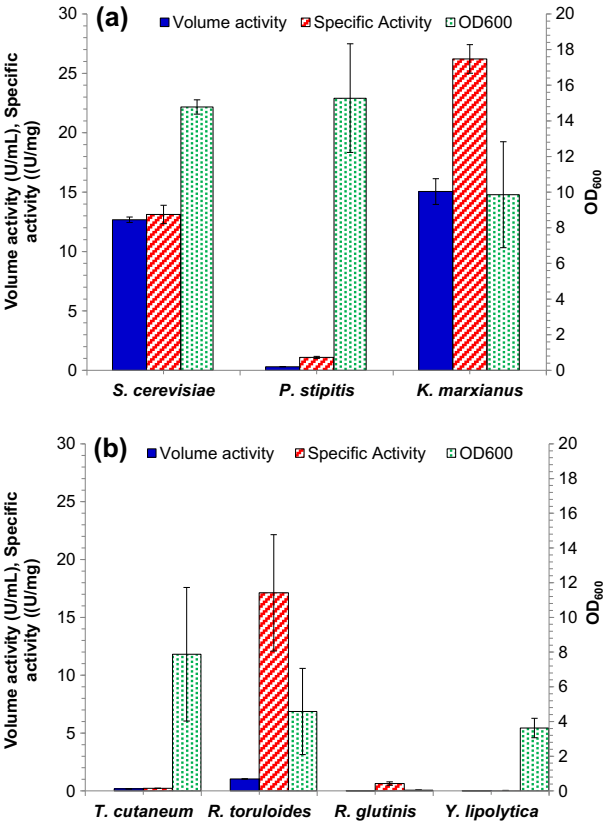



**Fig. 6** Comparison of inulin hydrolysis enzyme(s) among different yeast strains. **a** Ethanol production yeasts. **b** Oleaginous yeasts. Culture conditions—pH at 5.0, 30 °C, 180 rpm, for 24 h

relatively stable until inulin reached 50 g/L (Fig. 8c). The cell growth was roughly the same within the first 48 h at varied inulin concentrations, but the excessive inulin provided more carbon for the late cell growth. On the other hand, the lipid increased to 3.97 g/L from 1.12 g/L when inulin increased to 80 g/L from 10 g/L. The carbon/nitrogen ratio (C/N ratio) facilitated the cell growth constantly but the lipid accumulation had its maximum C/N ratio. The dry cell weight reached the highest level of 16.34 g/L at a C/N ratio of 73 in the experimental range, while the lipid titer reached the maximum of 4.79 g/L at a C/N ratio of 137 (Fig. 8d). The lipid maximum yield is 0.30 g/g glucose theoretically [35], while integrating all of the data in our study, the microbial lipid produced per gram of consumed sugar was low; it may be caused by the following reasons: the lipid production or cell culture condition was not optimized; the lipid production needs some other nutrients, trace elements, and so on; and the metabolism of *T. cutaneum* CGMCC 2.1374 needs to regulate or optimization. More studies are needed to overcome these problems.

The lipid composition was analyzed by extracting the lipids from cells, then transmethylated and analyzed. The major fatty acids included 15.4 % of palmitate (C16:0), 8.5 % of stearate (C18:0), 70.0 % of oleate (C18:1), and 5.4 % of linoleate (C18:2). The composition was similar to other oleaginous yeasts with inulin feedstock such as

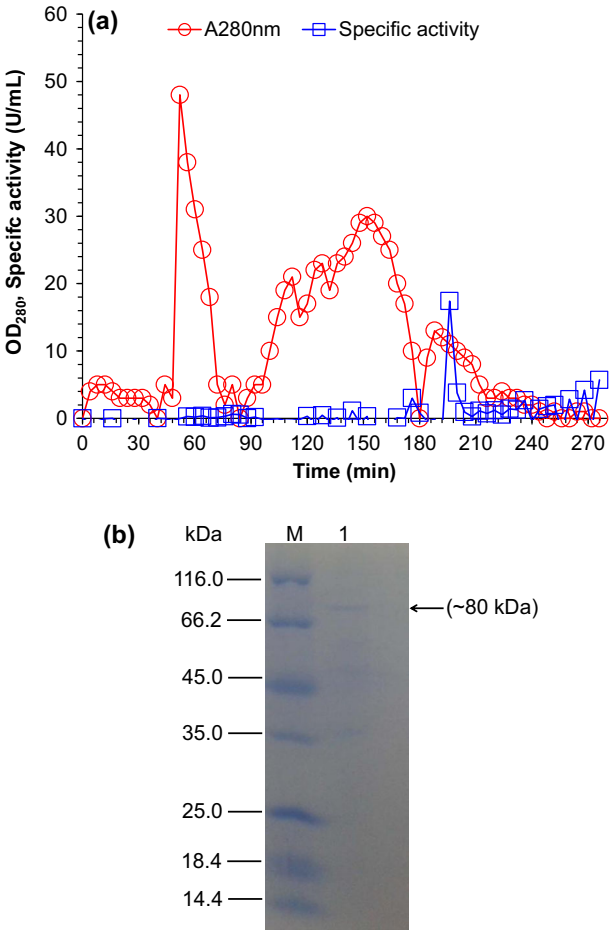



**Fig. 7** Purification of inulin hydrolyzing enzyme(s) in *T. cutaneum* CGMCC 2.1374. **a** Protein purification by Sephadex G-100 column chromatography. **b** SDS-PAGE of inulin hydrolyzing enzyme. *Lane M*: protein molecular markers, *Lane 1*: inulin hydrolyzing enzyme band

*R. mucilaginosa* [3], *Cryptococcus albidus* [36], and *Y. lipolytica* [37] as well as similar to that of vegetable oil [38].

This study demonstrated that the CBP of microbial lipid can be effectively produced from inulin directly by culturing oleaginous yeast *T. cutaneum* CGMCC 2.1374, resulting in significant lipid accumulated intracellularly, like the CBP bioethanol production by *S. cerevisiae* with Jerusalem artichoke tubers or microbial lipid production directly by *R. toruloides* with inulin. This result suggests that the CBP property on utilizing inulin substrate might cover a wide species of yeasts from ethanol fermenting strains to oleaginous strains. In this study, we characterized the inulin hydrolyzing enzyme properties of *T. cutaneum* CGMCC 2.1374. The putative enzyme responsible for inulin hydrolysis was found to be similar to sucrase or invertase in molecular weight. However, the accurate identification of the inulin hydrolyzing gene(s) was still not solved; because of lack of genome information of *T. cutaneum*, the cloning of the encoding genes was tested but not successful. It

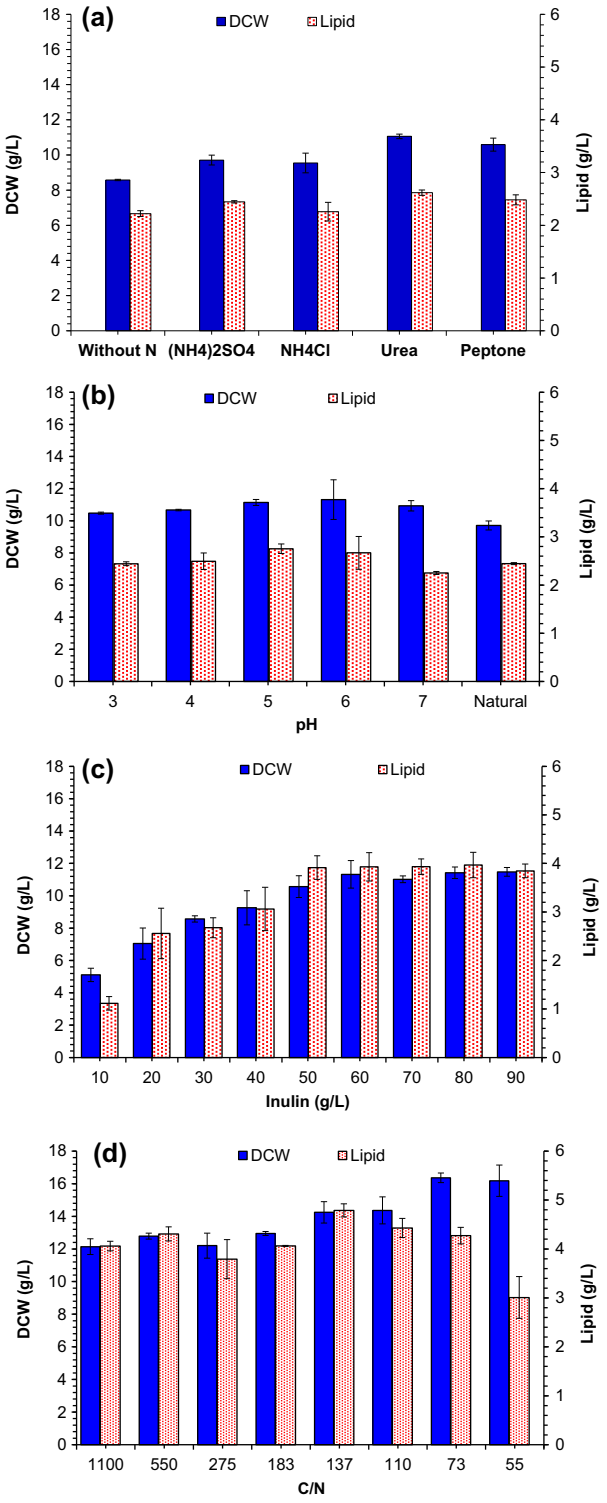



**Fig. 8** Microbial lipid production of *T. cutaneum* 2.1374 using inulin under different conditions. **a** Varied nitrogen sources; **b** varied initial pH; **c** varied inulin concentration; and **d** varied C/N ratio. Culture conditions—pH at 5.0, 30 °C, 180 rpm, for 120 h

is worth trying in the future for further identification of inulin hydrolyzing enzyme(s) and metabolic modification for enhanced CBP lipid fermentation.

## Conclusions

*T. cutaneum* CGMCC 2.1374 was found to utilize inulin directly for cell growth and lipid accumulation without the hydrolysis step of inulin, which suggested that it had the capability of inulin hydrolysis and express inulinase-like enzyme(s). The molecular weight of the enzyme was close 80 kDa, similar to invertase in *S. cerevisiae*. The properties of the potential inulin hydrolyzing enzyme of *T. cutaneum* CGMCC 2.1374 were investigated. *T. cutaneum* CGMCC 2.1374 produced a lipid titer of 4.79 g/L at the medium C/N ratio of 137. The fatty acid of microbial lipid produced by *T. cutaneum* CGMCC 2.1374 from inulin was composed by C16 and C18 two-carbon chain length fatty acid, and the unsaturated fatty acid content was 76.1 % and could be used as raw material for biodiesel production.

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