Bioprocess and Biosystems Engineering
https://doi.org/10.1007/s00449-025-03220-7

RESEARCH PAPER

From wheat straw to soluble yeast extract: enhanced mycoprotein production by adaptively evolved *Trichosporon cutaneum* and fermentation pH shifting

Chaolong Qu[1] · Dayu Ren[1] · Qi Liu[1] · Bin Zhang[1] · Jie Bao[1]

Received: 1 July 2025 / Accepted: 31 July 2025
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2025

Abstract

Single-cell protein (SCP) produced by yeast using low-cost agricultural wastes shows great potential as an alternative protein source for animal and human nutrition. In this study, we developed an adaptive evolution method coupled with centrifugal fractionation and pH shifting to enhance SCP production by *Trichosporon cutaneum* from wheat straw. During the adaptive evolution, the culture pH was shifted from 5.0 to 7.0, which is more favorable for SCP accumulation of *T. cutaneum*. The finally obtained *T. cutaneum* CL160 exhibited a 109.2% increase in SCP content compared to the parental strain. The DCW and SCP titer of *T. cutaneum* CL160 reached 48.6 ± 1.5 g/L and 14.2 ± 1.1 g/L using wheat straw clarified hydrolysate by batch fermentation. Fed-batch fermentation using wheat straw-derived syrup further improved DCW and SCP titer to 124.2 g/L and 32.6 g/L. Further attempts were performed to prepare soluble yeast extract from lignocellulose-derived SCP by cell autolysis. This yeast extract served as an effective nitrogen source for lactic acid fermentation by *Pediococcus acidilactici*, achieving 83.2 ± 1.1 g/L lactic acid titer and 45×10^9/mL CFU value, comparable to commercial yeast extract. This study demonstrates the conversion of waste lignocellulosic feedstocks into sustainable SCP and soluble yeast extract, presenting an innovative strategy for the valorization of non-food lignocellulosic feedstocks.

Keywords Single cell protein · Lignocellulose · Adaptive evolution · pH shifting · Yeast extract

Introduction

Single-cell protein (SCP) produced by bacteria, fungi, algae, and yeast offers an alternative protein source for animal and human nutrition [1]. Yeast emerges as the most promising microbial cell factory for SCP production due to its rapid growth rate and sugars assimilation, high protein content, adaptability to diverse substrates, favorable amino acid composition, excellent flocculation ability, and low pathogenicity [2]. Costs of substrates generally account for 45–75% of the total costs of SCP production [3]. Leveraging the abundant and low-cost lignocellulosic feedstocks as a carbon source represents a viable approach to reducing SCP production costs [4].

Lignocellulosic feedstocks should undergo a harsh pretreatment process to overcome the natural recalcitrance of structure before enzymatic hydrolysis to release fermentable sugars [5]. The phenolic inhibitors generated during the pretreatment are difficult to completely remove. Some yeasts such as *Rhodosporidium toruloides, Rhodotorula glutinis,* and *Yarrowia lipolytica* are sensitive to these phenolic inhibitors [6], while *Trichosporon cutaneum* exhibited remarkable tolerance to lignocellulose-derived inhibitors [7].

T. cutaneum has been historically utilized for SCP production using steam exploded hemicellulose hydrolysate [8]. Our recent study engineered a *T. cutaneum* MP11 strain with super large cell volume [9]. *T. cutaneum* MP11 exhibited excellent capability for producing SCP using lignocellulosic feedstocks by simultaneous saccharification and co-fermentation (SSCF) at cellulase optimal working pH 5.0 [10]. Although SSCF can alleviate the high concentration sugars-induced inhibition on cellulase activity and achieve high SCP yield, the resulting mixture of lignin residues and

✉ Bin Zhang
binzh@ecust.edu.cn

✉ Jie Bao
jbao@ecust.edu.cn

[1] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

Published online: 07 August 2025

cell mass complicates the downstream separation. A recent study showed that *T. cutaneum* produced more SCP at pH 7.0 rather than at pH 5.0 [11]. Consequently, the current SCP production process by *T. cutaneum* using lignocellulosic feedstocks needs to be updated, recommending the adoption of separate hydrolysis and fermentation (SHF) at fermentation pH 7.0 to enhance SCP production and simplify the recovery process.

Nevertheless, the production performances of SCP by yeasts in clarified lignocellulosic hydrolysate remain relatively low [12]. An efficient approach should be developed to enhance the SCP production of *T. cutaneum* using lignocellulosic hydrolysate. Density gradient centrifugation has been widely employed for separating different cell types, sub-cellular compartments, and macromolecular complexes based on their differential buoyant densities [13, 14]. This technique has proven effective in isolating oleaginous yeasts or algae mutants with higher lipid content, which exhibit lower density and thus accumulate in the upper layer [9, 15–17]. Even simple gravimetric enrichment can facilitate the isolation of microalgal mutants—those with higher starch content (higher density cells settling in bottom layer) and those with higher lipid content (lower density cells floating in upper layer) [18].

In this study, adaptive evolution method coupled with centrifugal fractionation and pH shifting was employed to enhance the SCP production of *T. cutaneum*. After centrifugation, the mutants with higher cell density were enriched in the bottom and transferred to the next round culture. During the adaptive evolution, the pH was systematically shifted from 5.0 to 7.0, which is more optimal for SCP production of *T. cutaneum*. The finally obtained *T. cutaneum* CL160 strain after 160 transfers showed significantly improved SCP production using wheat straw clarified hydrolysate compared to the parental strain. We further investigated the method for preparing soluble yeast extract from lignocellulose-derived SCP and evaluated its potential as an alternative complex nitrogen source for bacterial lactic acid fermentation. This study successfully achieved the transformation of waste lignocellulosic feedstocks to sustainable soluble yeast extract, offering an innovative strategy for the valorization of non-food lignocellulosic feedstocks.

Materials and methods

Feedstock, enzymes, and reagents

The raw wheat straw was harvested in May 2021 in Nanyang City, Henan Province, China. It was coarsely chopped through a 10 mm mesh. According to the protocol of the National Renewable Energy Laboratory (NREL), the main components of the wheat straw included cellulose 35.6 ± 0.4% (w/w), xylan 24.3 ± 0.2% (w/w), lignin 18.7 ± 0.1% (w/w), and ash 9.7 ± 0.3% (w/w).

The commercial cellulase Cellic CTec 3.0 was purchased from Novozymes (Beijing, China). *β*-Mannanase (5×10^4 U/g) and neutral protease (5×10^4 U/g) were purchased from Sunson Biotechnology Development Co., Ltd (Beijing, China). The commercial lyticase complex ($35–40\times10^4$ U/g) was purchased from Doing-Higherbio Tech Co., Ltd (Nanning, China). The yeast extract for industrial use was purchased from Angel Yeast Co., Ltd (Yi Chang, China). All the reagents were purchased from Titan platform (Shanghai, China).

Microorganisms and medium

The pretreated wheat straw hydrolysate was biodetoxified by *Paecilomyces variotii* FN89 (CGMCC No. 17665) [19]. *P. variotii* FN89 was activated and preserved on PDA plates, and cultured in liquid synthetic medium composed of 2 g/L KH_2PO_4, 0.5 g/L $CaCl_2$, 1 g/L yeast extract, 1 g/L $(NH_4)_2SO_4$, 1 g/L $MgSO_4{\cdot}7H_2O$, and 20 g/L glucose.

The engineered *Trichosporon cutaneum* MP11 (CGMCC No. 20481), derived from *T. cutaneum* ATCC 20271 [9], was used as the starting strain for adaptive evolution. *T. cutaneum* MP11 was activated and cultured in YPD medium. The synthetic medium for SCP fermentation contained 90 g/L glucose and 45 g/L xylose as carbon sources. The nutrients for SCP production by *T. cutaneum* using refined sugars or lignocellulosic hydrolysate included 1 g/L KH_2PO_4, 1 g/L $MgSO_4{\cdot}7H_2O$, 24 g/L $(NH_4)_2SO_4$, and 1 g/L yeast extract.

The engineered *Pediococcus acidilactici* ZY271 (CGMCC No. 13611) was used for lactic acid fermentation to evaluate the potential of yeast extract prepared from cellulosic SCP as an alternative complex nitrogen source. *P. acidilactici* ZY271 was activated and cultured in simplified MRS medium containing 20 g/L glucose, 10 g/L peptone, 10 g/L yeast extract, 5 g/L CH_3COONa, 2 g/L $C_6H_{14}N_2O_7$, 2 g/L K_2HPO_4, 0.58 g/L $MgSO_4{\cdot}7H_2O$, and 0.25 g/L $MnSO_4{\cdot}H_2O$. The medium for lactic acid fermentation included 95 g/L glucose, 45 g/L xylose, 50 g/L $CaCO_3$, 25 g/L yeast extract (industrial grade), 10 g/L $(NH_4)_2SO_4$, and 0.25 g/L $MnSO_4{\cdot}H_2O$.

Wheat straw biorefinery process and syrup preparation

Wheat straw was pretreated using diluted sulfuric acid as catalyst in a 20 L reactor [20]. The initial solids content of pretreatment was ~ 70% (w/w). The sulfuric acid dosage was 50 mg/g dry wheat straw. The pretreatment was conducted at 175 °C, 50 rpm for 5 min. The pretreated wheat straw was discharged from the bottom outlet of the reactor and then neutralized with $CaCO_3$ powder to pH ~ 5.0. The neutralized

wheat straw was enzymatically hydrolyzed at 30% (w/w) solids loading in a 5 L bioreactor equipped with helical impeller with the cellulase dosage of 4 mg protein/g substrate (dry weight). The enzymatic hydrolysis was performed at 50 °C, 200 rpm for 72 h. The obtained hydrolysate contained 130.0 g/L glucose, 65.7 g/L xylose, 13.8 g/L acetate, 1.0 g/L furfural, and 0.5 g/L 5-hydroxymethylfurfural (HMF).

The wheat straw hydrolysate was transferred to a 3 L fermenter for submerged liquid biodetoxification. *P. variotii* FN89 was cultured in liquid synthetic medium at 37 °C, 250 rpm for 18 h as the seed, and then inoculated into the hydrolysate at a 10% (v/w) ratio. The submerged liquid biodetoxification was carried out at 37 °C, 1 vvm, 750 rpm. The biodetoxification was finished when the pH value of the hydrolysate began to decline [21]. The biodetoxified hydrolysate contained 118.5 g/L glucose, 56.2 g/L xylose, and 2.2 g/L acetate. The biodetoxified hydrolysate was centrifuged at 8000 × g for 15 min to remove the solid residues. The clarified hydrolysate was used for SCP batch fermentation. The clarified hydrolysate can be further concentrated by rotary evaporation at 120 °C for 50 min, producing the syrup containing 355.2 g/L glucose, 166.9 g/L xylose, and 7.4 g/L acetate. The wheat straw-derived syrup was used for fed-batch fermentation.

Adaptive evolution coupled with centrifugal fractionation and pH shifting

T. cutaneum MP11 was cultured in 50 mL synthetic medium at 30 °C and 200 rpm for 4 days, and then subjected to adaptive evolution coupled with centrifugal fractionation and pH shifting to enhance its SCP accumulation. The broth of 20 mL was centrifuged. The bottom cell pellets were resuspended using 20 mL fresh medium as the seed and inoculated into 50 mL fresh medium at a 10% (v/v) ratio for the next round of culture. The adaptive evolution coupled with centrifugal fractionation was conducted for a total of 160 transfers, divided into three phases. Phase I started from parental *T. cutaneum* MP11 to the 52nd transfer. The centrifugal force gradually increased from 4000 to 6000 × g. Phase II started from the 53rd transfer to the 135th transfer. The centrifugal force alternated between 6000 × g and 30,000 × g. Phase III started from the 136th transfer to the 160th transfer. The centrifugal force was 30,000 × g. The centrifugal time was 5 min. During Phase III, the broth pH was adjusted to 7.0 every 24 h by adding 5 M NaOH solution.

Batch and fed-batch fermentation for SCP production

T. cutaneum was activated on PDA plates at 30 °C for 48 h. One single colony was picked up, inoculated into fresh liquid YPD medium, and cultured at 30 °C, 200 rpm for 24 h as the seed. The fermentation was conducted in a 3 L fermenter containing 1 L fresh liquid synthetic medium or clarified hydrolysate. The seed was inoculated at a 10% (v/v) ratio. The fermentation conditions were set at 30 °C, 1 vvm, 600 rpm. The fermentation pH was maintained at 5.0 or 7.0 by automatically adding 2 M H_2SO_4 and 5 M NaOH solution. For the fed-batch fermentation, the fermentation process involved feeding the lignocellulose-derived syrup three times at 4 h intervalsbetween 40 and 48 h.

Soluble yeast extract preparation from fermented SCP

The broth was centrifuged, and the wet cell mass was washed twice. The soluble yeast extract was prepared by salt-or-enzyme-mediated cell autolysis at 20% (w/v) cell mass content. For the salt-mediated cell autolysis, the wet cell mass was mixed with 0%, 2%, 4%, and 6% (w/v) NaCl solution. For the enzyme-mediated cell autolysis, the β-glucanase, neutral protease, and commercial lyticase were added into the wet cell mass at the dosage of 800, 1200, 1600, and 2000 U/g (dry cell mass). The pH values of the mixtures were adjusted to the optimal pH for the enzymes (pH 4.0 for β-glucanase, pH 7.0 for neutral protease, and pH 5.5 for commercial lyticase, respectively). The mixture was shaken at 50 °C, 200 rpm for 24 h, and then centrifuged to remove the cell debris. The supernatant was used as an alternative complex nitrogen source to replace industrial yeast extract for lactic acid production by *P. acidilactici* ZY271. The lactic acid fermentation was conducted at 42 °C, 150 rpm for 48 h.

Calculations

The fermentation protein content and titer were measured by semiautomatic Kjeldahl apparatus (PeiOu Analysis Instrument, Shanghai, China). The protein content (w_1, %) was calculated according to Eq. (1) as follows:

$$w_1 = \frac{c_1 \times (v_1 - v_0) \times 14 \times 6.25}{m_1} \times 100\% \quad (1)$$

where c_1 (mol/L) is the concentration of standard HCl solution, which is 0.05 mol/L; v_1 (mL) is the usage of standard HCl solution; v_0 (mL) is the usage of standard HCl solution for the titration of the blank control; 14 is the molar mass of nitrogen; 6.25 is the coefficient for conversion of nitrogen to protein; m_1 (g) is dry cell weight.

The SCP titer (c, g/L) is calculated according to Eq. (2) as follows:

$$\mathrm{C} = \frac{w_1 \times m_1}{v_3} \quad (2)$$

where w_1 (%) is protein content; m_1 is dry cell weight; v_3 is the broth volume.

The yield (%) of soluble yeast extract from SCP was calculated according to Eq. (3) as follows:

$$\text{Yield}\ (\%) = \frac{c_2 \times v_2}{m_2 \times w_1} \tag{3}$$

where c_2 (g/L) is the protein concentration in the supernatant after hydrolysis; v_2 (mL) is the supernatant volume; m_2 (g) is the dry cell mass; w_1 (%) is the protein content.

Analytical methods

Dry cell mass (DCW) was measured by differential weighing method. The concentrations of glucose, xylose, acetic acid, furfural, 5-hydroxymethylfurfural (5-HMF), and lactic acid were analyzed using a HPLC system equipped with a Bio-Rad Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) and an RID-10A refractive index detector (Shimadzu, Kyoto, Japan). The mobile phase was 5 mM sulfuric acid (H_2SO_4) solution with a flow rate of 0.6 mL/min. The lipid was determined using methanol-chloroform methods [22]. The main cell wall components' contents, including glucan, mannan, and chitin, were measured by reported methods [23, 24].

Results and discussions

Adaptive evolution coupled with centrifugal fractionation and pH shifting enhanced SCP accumulation in *T. cutaneum*

Proteins (density > 1.10 g/mL) have relatively higher density compared with lipids (0.88–0.90 g/mL) [14, 15]. Consequently, the cells with higher lipid content generally exhibit lower cell density, while those with higher SCP content exhibit higher cell density. After centrifugation, the mutants with increased SCP content and higher lipid content would be separately enriched in the bottom and upper layers, which can be easily isolated by pipette. Through iterative centrifugal fractionation, the higher SCP-producing mutants were continuously screened, transferred, and cultured (Fig. 1a). The adaptive evolution comprising 160 serial cycles of culture, centrifugation, fractionation, and transfer, systematically divided into three stages with different centrifugal forces (Figs. 1b, 2a). The changes in SCP contents of *T. cutaneum* during the adaptive evolution are shown in Fig. 2a. The results show that stage I effectively improved the SCP content from ~ 12% (w/w) to ~ 25% (w/w). However, increasing the centrifugal force from 6000 to 30,000 g failed to further improve the SCP content in stage II. pH shifting strategy

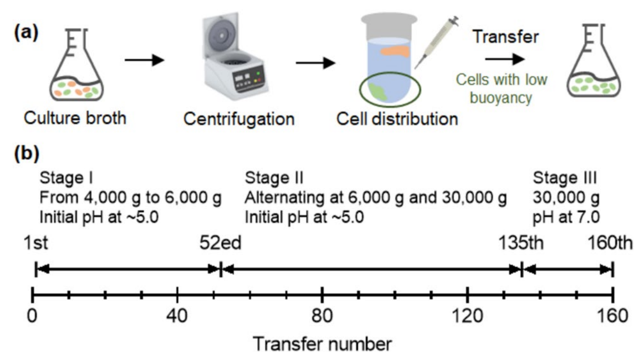


Fig. 1 Adaptive evolution coupled with centrifugal fractionation and pH shifting to enhance SCP production of *T. cutaneum*. **a** The schematic diagram of adaptive evolution with centrifugal fractionation. **b** The changes of centrifugal forces and culture pH values during the adaptive evolution. The centrifugal time was 5 min

was thus adopted in stage III (from 136th transfer to 160th transfer) of the adaptive evolution (Fig. 1a). Given that pH plays an important role in the growth and SCP production of microbes [25, 26], and Sun et al. reported that the optimal pH was 7.0 for cell growth and sugar metabolism of *T. cutaneum* [11]. The pH was therefore controlled at 7.0 in stage III. The adaptive evolution coupled with centrifugal fractionation and pH shifting further improved the SCP content to ~ 30% (w/w). The final obtained cell pellets were spread on a PDA plate. A single colony was isolated and named *T. cutaneum* CL160.

The parental strain and evolved *T. cutaneum* CL160 were cultured at pH 5.0 and pH 7.0 for 4 days, respectively, using 90 g/L glucose and 45 g/L xylose as carbon sources (Figs. 2, 3). After the centrifugation of broth at 6000 g for 5 min, the cell mass distribution showed that the parental strain supernatant remained turbid, whereas the *T. cutaneum* CL160 supernatant appeared clear (Fig. 2b), indicating that the cell density of *T. cutaneum* CL160 was higher than that of the parental strain. The morphological observation by optical microscope showed that the parental cells exhibited an elongated rod shape with larger cell volume, and the lipid bodies filled the intracellular space (Fig. 2b). In contrast, the cell volume of *T. cutaneum* CL160 was significantly reduced, accompanied by a decrease in intracellular lipid bodies, indicating that more intracellular space became available for SCP accumulation. *T. cutaneum* CL160 showed a 109.2% increase in SCP content and a 58.5% decrease in lipid content culture compared to that of the parental strain at pH 5.0 (Fig. 2c). These findings indicated that this long-term adaptive evolution redirected the carbon flux from lipid storage toward protein biosynthesis. The contents of the main components of the yeast cell wall, including glucan, mannan, and chitin, were measured in the parental and evolved strains [27]. The results showed that the evolved strain exhibited 6.3-fold, 12.4-fold, and 10.7-fold increases in glucan,

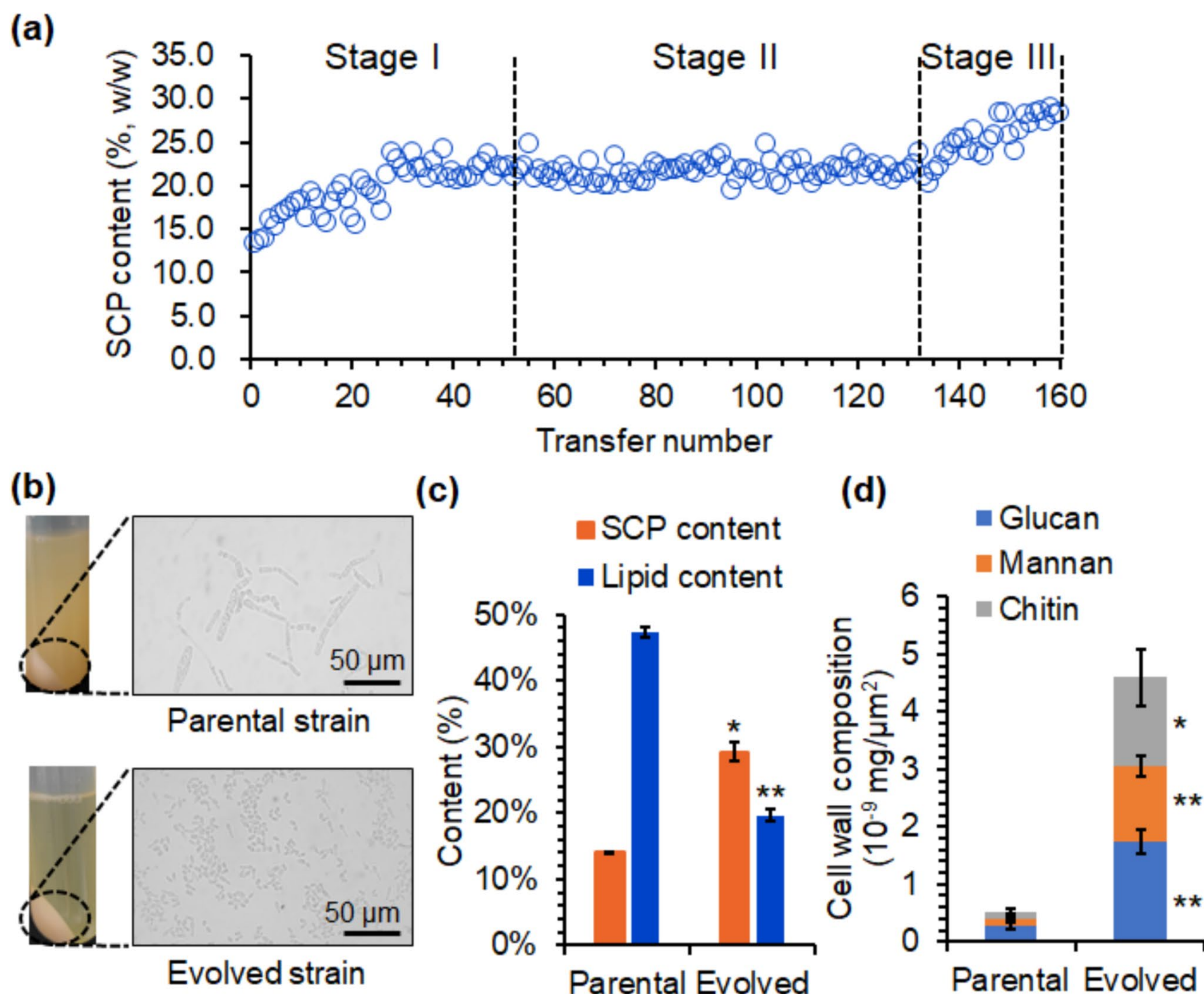


Fig. 2 Comparisons of cell mass distribution, SCP content, and lipid content between parental and evolved strains. **a** SCP content during the adaptive evolution. **b** Cell mass distribution after the centrifugation of broth at 6000 g for 5 min. **c** SCP content and lipid content of parental and evolved strains. **d** Main cell wall compositions of parental and evolved strains. The parental strain was cultured without pH control for 4 days, and *T. cutaneum* CL160 was cultured at pH 7.0 for 4 days. Variations were considered statistically significant at $*P<0.05$ and $**P<0.01$. The determinations of SCP content and cell wall composition were performed in triplicate. The error bar represents the standard deviation

mannan, and chitin content, respectively, compared to the parental strain (Fig. 2d). The strain evolved reinforced the cell wall structure to withstand the iterative centrifugal force stress. However, the more rigid cell wall structure would have negative effects on cell autolysis and lysis process.

The detailed fermentation profiles of sugars consumption, cell growth, lipid, and SCP production by parental strain and *T. cutaneum* CL160 using mixed glucose and xylose at pH 5.0 and 7.0 are shown in Fig. 3. *T. cutaneum* exhibited the ability to co-utilize all the glucose and xylose, which is consistent with a previous report [28]. The sugars (glucose and xylose) consumption rates during the first 48 h of fermentation were calculated. At pH 5.0, the parental strain exhibited a higher sugar consumption rate (2.6 g/L/h) than the evolved strain (1.5 g/L/h). At pH 7.0, the evolved strain exhibited an increased sugar consumption rate (2.0 g/L/h), whereas the parental strain showed a lower rate (1.4 g/L/h). At pH 5.0, the parental strain showed stronger cell growth; the highest DCW reached 37.7 ± 2.3 g/L at 48 h (Fig. 3a). But the highest SCP titer of the parental strain was only 7.7 ± 1.3 g/L at 48 h (Fig. 3b). The increase of pH from 5.0 to 7.0 significantly improved the DCW of *T. cutaneum* CL160, which increased from 27.3 ± 1.3 g/L (72 h, pH 5.0) to 46.1 ± 2.2 g/L (96 h, pH 7.0) (Figs. 3a, c). The SCP titer of *T. cutaneum* CL160 reached 13.5 ± 0.3 g/L at 96 h (pH 7.0), which was 3.2 times higher than that of the parental strain

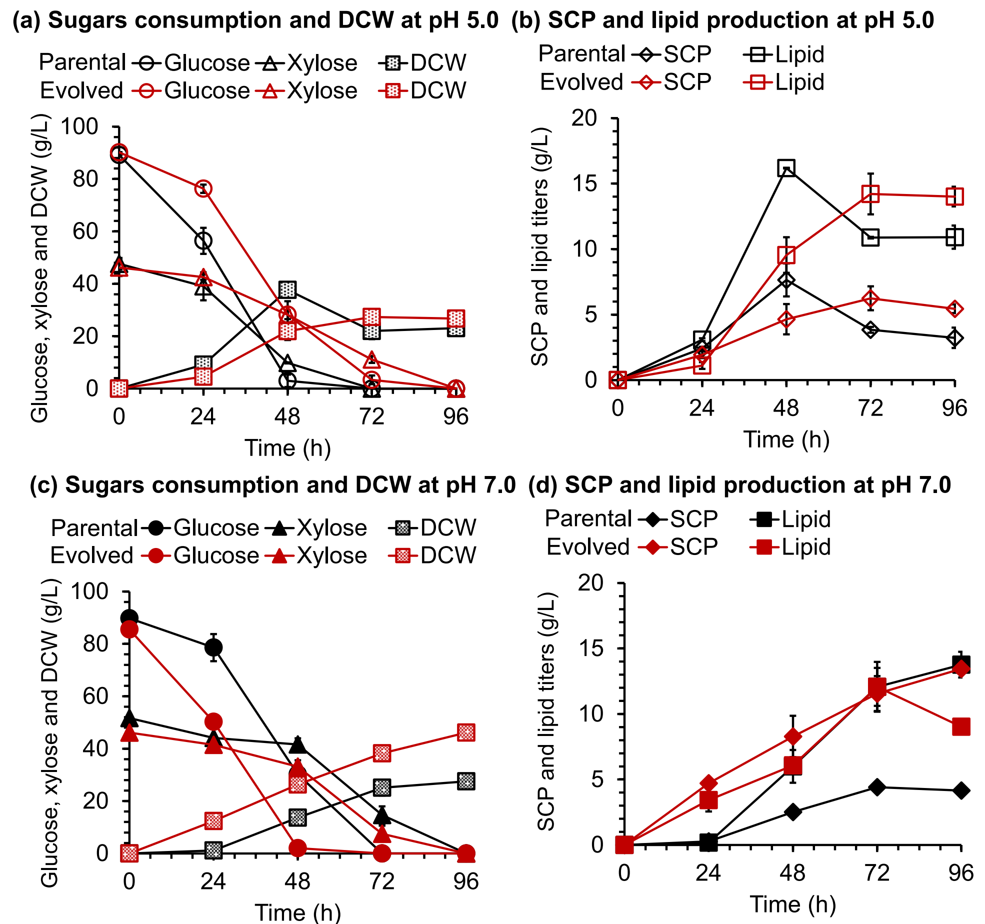


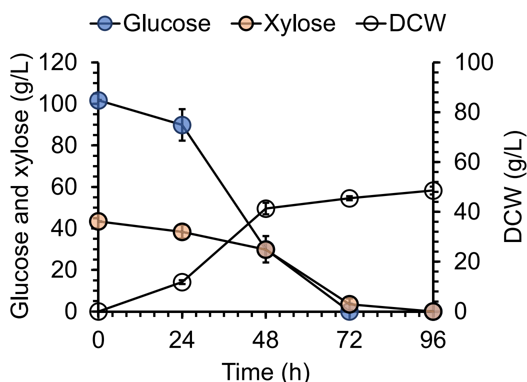
Fig. 3 SCP fermentation at different pH in synthetic medium by parental and *T. cutaneum* CL160 strains. Sugars consumption and DCW at pH 5.0 (**a**); SCP and lipid production at pH 5.0 (**b**); sugars consumption and DCW at pH 7.0 (**c**); SCP and lipid production at pH 7.0 (**d**). Conditions: 600 rpm, 30 °C, 1 vvm. pH was controlled at 5.0 or 7.0. Each experiment was performed in triplicate. The error bar represents the standard deviation

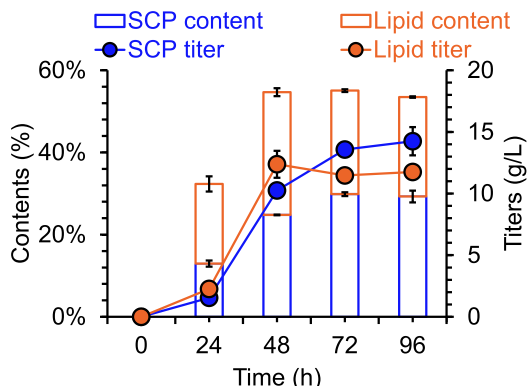
(4.2 ± 0.3 g/L) (Fig. 3d). Additionally, the lipid production of both the parental strain and *T. cutaneum* CL160 was lower at pH 7.0 than that at pH 5.0. The lipid was consumed by *T. cutaneum* CL160 after 72 h (Fig. 3d). These results indicated that this adaptive evolution method coupled with centrifugation fractionation and pH shifting drove a metabolic shift in *T. cutaneum*, favoring SCP production over lipid storage.

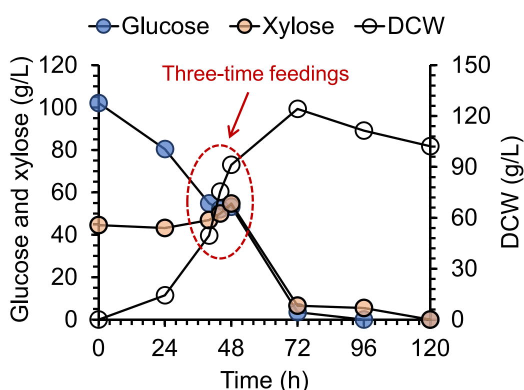
High-titer SCP production from wheat straw by *T. cutaneum* CL160

The wheat straw was diluted acid pretreated, enzymatically hydrolyzed, biodetoxified, and centrifuged to remove residual solids. *T. cutaneum* CL160 was further employed for SCP production using clarified wheat straw hydrolysate as a carbon source. All the glucose and xylose hydrolyzed from wheat straw were consumed by *T. cutaneum* CL160 (Fig. 4a), and the DCW and SCP titer reached 48.6 ± 1.5 g/L and 14.2 ± 1.1 g/L at 96 h (Fig. 4b), respectively, with the SCP content of 29.3% (w/w). The highest lipid titer reached 12.4 ± 1.1 g/L at 48 h, followed by a slight decline from 48 to 96 h.

The wheat straw clarified hydrolysate was evaporated to prepare the concentrated syrup and used for fed-batch fermentation. The fed-batch fermentation process involved three syrup feedings at 4 h intervals between 40 and 48 h (Fig. 4c). The syrup was fed at the ratio of 10% (v/v) of working volume. The highest DCW and SCP titers reached 124.2 g/L and 32.6 g/L at 72 h (Figs. 4c, d). Then the SCP titer decreased, while the lipid titer gradually increased. Zayed and Mostafa reported that young cells tended to accumulate high protein content while the mature cells would store high amounts of carbohydrates [29]. Kumar et al. further explained this phenomenon: the oleaginous yeasts would accumulate lipid at the expense of proteins when grown on lignocellulosic hydrolysate under limited nitrogen [30]. Nitrogen limitation is necessary for the accumulation of lipids by oleaginous yeasts [31]. In this study, the high cell mass generated by fed-batch fermentation would lead to the depletion of nitrogen, triggering the consumption of SCP and the production of lipid. Therefore, it is essential to control the nitrogen concentration during SCP fermentation [32]. Once the microorganisms have reached sufficient biomass, the SCP should be harvested promptly.







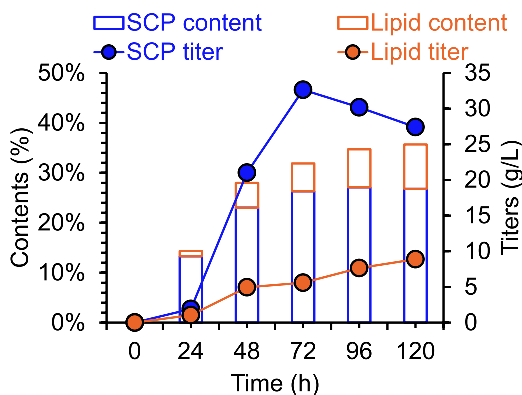


Fig. 4 SCP production by *T. cutaneum* CL160 using wheat straw. Sugars consumption and DCW (**a**), SCP and lipid production (**b**) by batch fermentation using 30% (w/w) solids loading wheat straw clarified hydrolysate. Sugars consumption and DCW (**c**), SCP and lipid production (**d**) by fed-batch fermentation using wheat straw-derived syrup. Conditions: pH 7.0, 600 rpm, 30 ℃, 1 vvm. The batch fermentation was performed in triplicate. The error bar represents the standard deviation

Table 1 Summary of single cell proteins produced from lignocellulosic feedstock

Strain	Feedstock	Titer (g/L)	Content (%, w/w)	Yield (g/g)	Productivity (g/L/h)	Source
T. cutaneum	Wheat straw	32.6	26.3	0.21	0.45	This study
Saccharomyces cerevisiae and *Candida utilis*	*Eucalyptus urophylla*	2.93	43.6	0.18	0.14	[33]
Candida tropicalis	Sugarcane bagasse	10.19	60.1	0.17	0.11	[34]
Rhodotorula mucilaginosa	Wastepaper residues	2.10	11.9	0.08	0.02	[35]
Candida utilis	Cotton stalk	5.74	n.d	0.23	0.08	[36]
Paecilomyces variotii	*Eucalyptus* chips	2.16	34.0	0.17	0.03	[37]
Candida tropicalis	Sugarcane bagasse	3.16	31.3	0.13	0.06	[38]
Penicillium janthinellum	Sugarcane bagasse	6.26	46.0	0.32	0.13	[39]
Chaetomium cellulolyticum	Aspen wood	3.86	21.4	0.23	0.19	[40]

n.d., not determined

The reported studies about the SCP production from lignocellulosic feedstock were summarized in Table 1. The SCP titer and productivity of evolved *T. cutaneum* CL160 are advanced in the reported studies. The SCP titer and yield of *T. cutaneum* CL160 are relatively mediocre because ~ 5 g/L lipid product was coproduced.

Fermentable soluble yeast extract preparation from lignocellulose-derived SCP

Although SCP is regarded as a promising alternative protein source, the main food safety risks in SCP, including high RNA content, toxic metabolites, and contamination of the microbial cultures with other microorganisms, prove insurmountable for its use as feed or food ingredient at present [41]. Using the disrupted microbial cells as an alternative complex nitrogen source in fermentation also presents a promising application scenario for SCP [42, 43]. For yeast cell disruption, the addition of salt or enzymes can attack the mannoprotein complex and glucan backbone of the yeast cell wall by autolysis or lytic enzymes, which are more commercially available at a reasonable cost compared to the mechanical and chemical methods [44].

The effects of different concentrations of NaCl solution (0–6%, w/w), different dosages (800–2000 U/g substrate) of β-glucanase, neutral protease and commercial lyticase on the yields of soluble yeast derived from *T. cutaneum* CL160 cells were evaluated (Fig. 5a–d). The results showed that the addition of 1600 U/g neutral protease or 2000 U/g complex lyticase significantly improved the extraction efficiency of soluble yeast extract from SCP, with the maximum yield reaching $87.3 \pm 0.9\%$ and $93.8 \pm 3.8\%$ of the cellulosic SCP (Fig. 5c, d).

Lactic acid bacterium generally requires complex nutrients for cell growth and metabolism owing to its incomplete biosynthesis pathway [45]. Although yeast extract as complex nitrogen is commonly used in lactic acid fermentation, it is not economically favorable for industrial lactic acid production [46]. Our previous studies attempted to use hydrolyzed plant protein by acid or enzyme as an alternative complex nitrogen to yeast extract; however, the lactic acid titer and cell viability using plant proteins were lower than the levels using yeast extract [47, 48]. The prepared yeast extract from *T. cutaneum* CL160 using wheat straw as a carbon source was further used for lactic acid production by *P. acidilactici* ZY271 (Fig. 5e, f). The control group used 20 g/L industrial grade yeast extract as a complex nitrogen source, and the lactic acid titer (48 h) and CFU value (24 h) reached 84.8 ± 0.4 g/L and 47×10^9/mL, respectively. Different concentrations of soluble yeast extract prepared from *T. cutaneum* CL160 were used for lactic acid fermentation. The results showed that the lactic acid titer and CFU value reached 83.2 ± 1.1 g/L and 45×10^9/mL with the usage of 20 g/L soluble yeast extract from *T. cutaneum* CL160. Further increasing the dosage of soluble yeast extract from *T. cutaneum* CL160 did not enhance the lactic acid titer but significantly improved the cell growth of *P. acidilactici* ZY271. These results suggested that soluble yeast extract from *T. cutaneum* CL160 using wheat straw as a carbon source contained enough nutritional compounds for cell growth as well as lactic acid production by lactic acid bacteria. However, further concentration and drying processes are required to enable the commercialization of this wheat straw-derived soluble yeast extract [32].

Conclusion

An adaptive evolution method coupled with centrifugal fractionation and pH shifting was employed to enhance the SCP production of *T. cutaneum* from wheat straw hydrolysate. The finally obtained *T. cutaneum* CL160 showed a 109.2% increase in SCP content and a 58.5% decrease in lipid content at pH 7.0 compared to that of the parental strain at pH 5.0. DCW and SCP titer of *T. cutaneum* CL160 reached 48.6 ± 1.5 g/L and 14.2 ± 1.1 g/L using 30% (w/w) solids loading wheat straw clarified hydrolysate. DCW and SCP titer reached 124.2 g/L and 32.6 g/L by fed-batch fermentation using wheat straw-derived syrup. The produced cellulosic SCP was further processed into soluble yeast extract, which was successfully employed as an alternative complex nitrogen source for bacterial lactic acid fermentation. This study demonstrates the conversion of waste lignocellulosic feedstocks into sustainable SCP and soluble yeast extract, presenting an innovative strategy for the valorization of non-food lignocellulosic feedstocks.

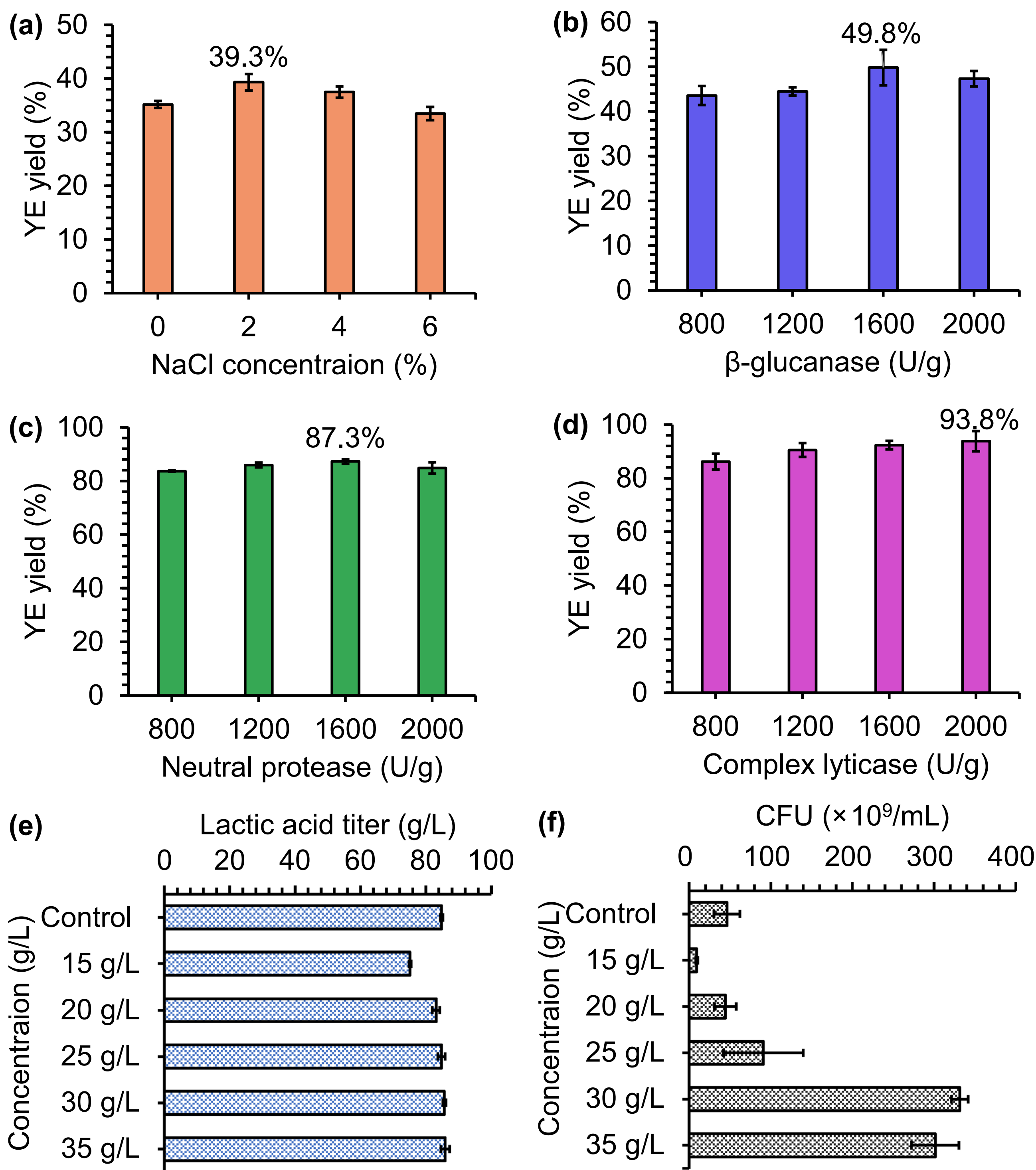


Fig. 5 Preparation of soluble yeast extract from *T. cutaneum* CL160 using wheat straw as carbon source. **a** Salt-mediated cell autolysis; **b** *β*-glucanase mediated cell autolysis at pH 4.0; **c** Neutral protease mediated cell autolysis at pH 7.0; **d** Commercial lyticase mediated cell autolysis at pH 5.5. Conditions: 20% (w/v) dry cell mass content, 50 °C, 200 rpm for 24 h. **e** Lactic acid titer by *P. acidilactici* ZY271 using soluble yeast extract from *T. cutaneum* CL160 as complex nitrogen source. Conditions: 42 °C, 150 rpm for 48 h. **f** Colony-forming unit (CFU) of *P. acidilactici* ZY271 at 24 h. Each experiment is performed in triplicate. The error bar represents the standard deviation

Acknowledgements This research was supported by the National Key R&D Program of China (2023YFA0914400), the National Natural Science Foundation of China (32301269), the Yangfan Project of Science and Technology Committee of Shanghai Municipality (23YF1409900), and the Key Technology R&D Program of the Science and Technology Commission of Shanghai Municipality (25HC2820200).

Author contributions C.Q., investigation, data curation, visualization, writing—original draft. D.R., investigation, methodology. Q.L., data curation, formal analysis, writing—original draft. B.Z., conceptualization, funding acquisition, writing—review and editing. J.B., supervision, project administration, funding acquisition, writing—review and editing.

Funding National Key R&D Program of China, 2023YFA0914400, National Natural Science Foundation of China, 32301269, Yangfan Project of Science and Technology Committee of Shanghai Municipality, 23YF1409900, Key Technology R&D Program of the Science and Technology Commission of Shanghai Municipality, 25HC2820200.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

References

1. Bertasini D, Binati RL, Bolzonella D, Battista F (2022) Single cell proteins production from food processing effluents and digestate. Chemosphere 296:134076. https://doi.org/10.1016/j.chemosphere.2022.134076
2. Yang F, Jin Z, Nawaz M, Xiao Y, Jiang Y, Hu J, Li J, Gao MT (2022) Oligosaccharides in straw hydrolysate could improve the production of single-cell protein with *Saccharomyces cerevisiae*. J Sci Food Agric 102:2928–2936. https://doi.org/10.1002/jsfa.11633
3. Sharif M, Zafar MH, Aqib AI, Saeed M, Farag MR, Alagawany M (2021) Single cell protein: sources, mechanism of production, nutritional value and its uses in aquaculture nutrition. Aquaculture 531:735885. https://doi.org/10.1016/j.aquaculture.2020.735885
4. Mahan KM, Le RK, Wells T Jr., Anderson S, Yuan JS, Stoklosa RJ, Bhalla A, Hodge DB, Ragauskas AJ (2018) Production of single cell protein from agro-waste using *Rhodococcus opacus*. J Ind Microbiol Biotechnol 45:795–801. https://doi.org/10.1007/s10295-018-2043-3
5. Zhang B, Liu X, Bao J (2023) High solids loading pretreatment: the core of lignocellulose biorefinery as an industrial technology—an overview. Bioresour Technol 369:128334. https://doi.org/10.1016/j.biortech.2022.128334
6. Zhang Y, Bao J (2022) Tolerance of *Trichosporon cutaneum* to lignin derived phenolic aldehydes facilitate the cell growth and cellulosic lipid accumulation. J Biotechnol 343:32–37. https://doi.org/10.1016/j.jbiotec.2021.09.009
7. Hu M, Wang J, Gao Q, Bao J (2018) Converting lignin derived phenolic aldehydes into microbial lipid by *Trichosporon cutaneum*. J Biotechnol 281:81–86. https://doi.org/10.1016/j.jbiotec.2018.06.341
8. Yinbo Q, Hongzhang C, Peiji G (1992) SCP production from steam exploded hemicellulose autohydrolysate by *Trichosporon cutaneum*. J Ferment Bioeng 73:386–389. https://doi.org/10.1016/0922-338X(92)90284-2
9. Liu Q, Lu M, Jin C, Hou W, Zhao L, Bao J (2022) Ultra-centrifugation force in adaptive evolution changes the cell structure of oleaginous yeast *Trichosporon cutaneum* into a favorable space for lipid accumulation. Biotechnol Bioeng 119:1509–1521. https://doi.org/10.1002/bit.28060
10. Zhang B, Ren D, Liu Q, Liu X, Bao J (2023) Coproduction of single cell protein and lipid from lignocellulose derived carbohydrates and inorganic ammonia salt with soluble ammonia recycling. Bioresour Technol 384:129345. https://doi.org/10.1016/j.biortech.2023.129345
11. Sun W, Zhang Z, Li X, Lu X, Liu G, Qin Y, Zhao J, Qu Y (2024) Production of single cell protein from brewer's spent grain through enzymatic saccharification and fermentation enhanced by ammoniation pretreatment. Bioresour Technol 394:130242. https://doi.org/10.1016/j.biortech.2023.130242
12. Aidoo R, Kwofie EM, Adewale P, Lam E, Ngadi M (2023) Overview of single cell protein: production pathway, sustainability outlook, and digital twin potentials. Trends Food Sci Technol 138:577–598. https://doi.org/10.1016/j.tifs.2023.07.003
13. Brakke MK (1951) Density gradient centrifugation: a new separation technique. J Am Chem Soc 73:1847–1848. https://doi.org/10.1021/ja01148a508
14. Eroglu E, Melis A (2008) "Density equilibrium" method for the quantitative and rapid in situ determination of lipid, hydrocarbon, or biopolymer content in microorganisms. Biotechnol Bioeng 102:1406–1415. https://doi.org/10.1002/bit.22182
15. Sung YJ, Choi HI, Lee JS, Hong ME, Sim SJ (2019) Screening of oleaginous algal strains from *Chlamydomonas reinhardtii* mutant libraries via density gradient centrifugation. Biotechnol Bioeng 116:3179–3188. https://doi.org/10.1002/bit.27149
16. Yamazaki H, Kobayashi S, Ebina S, Abe S, Ara S, Shida Y, Ogasawara W, Yaoi K, Araki H, Takaku H (2019) Highly selective isolation and characterization of *Lipomyces starkeyi* mutants with increased production of triacylglycerol. Appl Microbiol Biotechnol 103:6297–6308. https://doi.org/10.1007/s00253-019-09936-3
17. Liu L, Pan A, Spofford C, Zhou N, Alper HS (2015) An evolutionary metabolic engineering approach for enhancing lipogenesis in *Yarrowia lipolytica*. Metab Eng 29:36–45. https://doi.org/10.1016/j.ymben.2015.02.003
18. Hassanpour M, Abbasabadi M, Ebrahimi S, Hosseini M, Sheikhbaglou A (2015) Gravimetric enrichment of high lipid and starch accumulating microalgae. Bioresour Technol 196:17–21. https://doi.org/10.1016/j.biortech.2015.07.046
19. Zhang B, Khushik FA, Zhan B, Bao J (2021) Transformation of lignocellulose to starch-like carbohydrates by organic acid-catalyzed pretreatment and biological detoxification. Biotechnol Bioeng 118:4105–4118. https://doi.org/10.1002/bit.27887
20. He Y, Zhang J, Bao J (2014) Dry dilute acid pretreatment by co-currently feeding of corn stover feedstock and dilute acid solution without impregnation. Bioresour Technol 158:360–364. https://doi.org/10.1016/j.biortech.2014.02.074
21. Han T, Zhang B, Yang H, Liu X, Bao J (2023) Changes in pH values allow for a visible detection of the end point in submerged liquid biodetoxification during biorefinery processing. ACS Sustain Chem Eng 11:16608–16617. https://doi.org/10.1021/acssuschemeng.3c04986
22. Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509. https://doi.org/10.1016/s0021-9258(18)64849-5
23. Domer JE (1971) Monosaccharide and chitin content of cell walls of *Histoplasma capsulatum* and *Blastomyces dermatitidis*.

J Bacteriol 107(3):870–877. https://doi.org/10.1128/jb.107.3.870-877.1971
24. Manners DJ, Masson AJ, Patterson JC, Björndal H, Lindberg B (1973) The structure of a β-(1→6)-d-glucan from yeast cell walls. Biochem J 135:31–36. https://doi.org/10.1042/bj1350031
25. Rajput SD, Pandey N, Sahu K (2024) A comprehensive report on valorization of waste to single cell protein: strategies, challenges, and future prospects. Environ Sci Pollut Res 31:26378–26414. https://doi.org/10.1007/s11356-024-33004-7
26. Tian Y, Li J, Meng J, Li J (2023) High-yield production of single-cell protein from starch processing wastewater using co-cultivation of yeasts. Bioresour Technol 370:128527. https://doi.org/10.1016/j.biortech.2022.128527
27. Wang J, Li M, Zheng F, Niu C, Liu C, Li Q, Sun J (2018) Cell wall polysaccharides: before and after autolysis of brewer's yeast. World J Microbiol Biotechnol 34:137. https://doi.org/10.1007/s11274-018-2508-6
28. Wang Y, Yan R, Tang L, Zhu L, Zhu D, Bai F (2019) Dimorphism of *Trichosporon cutaneum* and impact on its lipid production. Biotechnol Biofuels 12:203. https://doi.org/10.1186/s13068-019-1543-3
29. Zayed G, Mostafa N (1992) Studies on the production and kinetic aspects of single cell protein from sugar cane bagasse saccharified by *Aspergillus Niger*. Biomass Bioenerg 3:363–367. https://doi.org/10.1016/0961-9534(92)90009-F
30. Kumar D, Singh B, Korstad J (2017) Utilization of lignocellulosic biomass by oleaginous yeast and bacteria for production of biodiesel and renewable diesel. Renew Sust Energ Rev 73:654–671. https://doi.org/10.1016/j.rser.2017.01.022
31. Bharathiraja B, Sridharan S, Sowmya V, Yuvaraj D, Praveenkumar R (2017) Microbial oil—a plausible alternate resource for food and fuel application. Bioresour Technol 233:423–432. https://doi.org/10.1016/j.biortech.2017.03.006
32. Salazar-López NJ, Barco-Mendoza GA, Zuñiga-Martínez BS, Domínguez-Avila JA, Robles-Sánchez RM, Ochoa MAV, González-Aguilar GA (2022) Single-cell protein production as a strategy to reincorporate food waste and agro by-products back into the processing chain. Bioengineering 9:623. https://doi.org/10.3390/bioengineering9110623
33. Zhang X, Zhao G (2022) Yeast cultivation for single-cell protein production using the carbohydrate hydrolysate of steam-exploded eucalyptus wood. Wood Res. 67:568–581. https://doi.org/10.37763/wr.1336-4561/67.4.568581
34. Magalhães CEB, Souza-Neto MS, Astolfi-Filho S, Matos ITSR (2018) *Candida tropicalis* able to produce yeast single cell protein using sugarcane bagasse hemicellulosic hydrolysate as carbon source. Biotechnol Res Inn 2:19–21. https://doi.org/10.1016/j.biori.2018.08.002
35. Campos-Valdez A, Kirchmayr MR, Barrera-Martínez I, Casas-Godoy L (2023) Sustainable production of single-cell oil and protein from wastepaper hydrolysate: Identification and optimization of a Rhodotorula mucilaginosa strain as a promising yeast. FEMS Yeast Res 23:1–12. https://doi.org/10.1093/femsyr/foad044
36. Zhong P, Chen P, Huo P, Ma L, Xu Z, Li F, Cai C (2025) Characterization of cotton stalk as a lignocellulosic feedstock for single-cell protein production. Bioresour Technol 417:131797. https://doi.org/10.1016/j.biortech.2024.131797
37. Almeida e Silva JB, de Mancilha IM, Vannetti MCD, Teixeira MA (1995) Microbial protein production by *Paecilomyces variotii* cultivated in *Eucalyptus* hemicellulosic hydrolyzate. Bioresour Technol 52:197–200. https://doi.org/10.1016/0960-8524(95)00029-E
38. Pessoa A, Mancilha IM, Sato S (1996) Cultivation of *Candida tropicalis* in sugarcane hemicellulosic hydrolyzate for microbial protein production. J Biotechnol 51:83–88. https://doi.org/10.1016/0168-1656(96)01572-6
39. Rao M, Varma AJ, Deshmukh SS (2010) Production of single cell protein, essential amino acids, and xylanase by *Penicillium janthinellum*. Bioresour 5:2470–2477. https://doi.org/10.15376/biores.5.4.2470-2477
40. Chahal DS, Moo-Young M, Vlach D (2004) Effect of physical and physicochemical pretreatments of wood for SCP production with *Chaetomium cellulolyticum*. Biotechnol Bioeng 23:2417–2420. https://doi.org/10.1002/bit.260231103
41. Lähteenmäki-Uutela A, Rahikainen M, Lonkila A, Yang B (2021) Alternative proteins and EU food law. Food Control 130:108336. https://doi.org/10.1016/j.foodcont.2021.108336
42. Moon S, Lee J, Song H, Cho J-H, Choi G-W, Seung D (2012) Characterization of ethanol fermentation waste and its application to lactic acid production by *Lactobacillus paracasei*. Bioprocess Biosyst Eng 36:547–554. https://doi.org/10.1007/s00449-012-0810-5
43. Xu Q, Bai F, Chen N, Bai G (2019) Utilization of acid hydrolysate of recovered bacterial cell as a novel organic nitrogen source for L-tryptophan fermentation. Bioengineered 10:23–32. https://doi.org/10.1080/21655979.2019.1586053
44. Liu D, Ding L, Sun J, Boussetta N, Vorobiev E (2016) Yeast cell disruption strategies for recovery of intracellular bio-active compounds—a review. Innov Food Sci Emerg Technol 36:181–192. https://doi.org/10.1016/j.ifset.2016.06.017
45. Pauli T, Fitzpatrick JJ (2002) Malt combing nuts as a nutrient supplement to whey permeate for producing lactic by fermentation with *Lactobacillus casei*. Process Biochem 38:1–6. https://doi.org/10.1016/S0032-9592(02)00038-9
46. Altaf M, Venkateshwar M, Srijana M, Reddy G (2007) An economic approach for L-(+) lactic acid fermentation by *Lactobacillus amylophilus* GV6 using inexpensive carbon and nitrogen sources. J Appl Microbiol 103:372–380. https://doi.org/10.1111/j.1365-2672.2006.03254.x
47. Zhang B, Han Y, Bao J (2024) Formulating the racemic lactic acids-free nitrogen ingredients from plant proteins for cellulosic chiral lactic acid fermentation. Ind Crop Prod 217:118821. https://doi.org/10.1016/j.indcrop.2024.118821
48. Zhang B, Wu L, Liu X, Bao J (2022) Plant proteins as an alternative nitrogen source for chiral purity l-lactic acid fermentation from lignocellulose feedstock. Fermentation 8:546. https://doi.org/10.3390/fermentation8100546

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.