ORIGINAL ARTICLE

Production of single cell protein from soy molasses using *Candida tropicalis*

Yurong Gao · Dapeng Li · Yang Liu

Abstract An effective bioprocess to produce single cell protein (SCP) from soy molasses using *Candida tropicalis* CGMCC 2.587 was developed. In a triangular flask, the optimal medium was 7 g/100 mL soy molasses concentration, 0.2 g/L yeast extract and 0.03 g/L $CaCl_2$, while the optimal environmental conditions were initial pH 5.5 and 30°C. Under these optimal conditions, maximum cell dry weight and total protein attained 10.83 and 6.11 g/L in a 10-L bioreactor. SCP contained 56.42%, and 5.28% crude protein and nucleic acid, respectively. SCP was rich in essential amino acids. Overall, 80.3, 98.2, 56.1 and 46.9% of total sugars, sucrose, raffinose and stachyose, respectively, were utilized in soy molasses. These results suggested that *C. tropicalis* CGMCC 2.587 might be applied effectively to produce SCP using soy molasses as a low-cost substrate.

Keywords Single cell protein · Soy molasses · *Candida tropicalis* · Low-cost substrate

Introduction

Single cell protein (SCP) is defined as a kind of protein source from pure or mixed microbial cultures including algae, yeasts, molds or bacteria for feeding animals and even humans (Rajoka et al. 2004). With the continuing expansion of the world's population, SCP has been used as an important protein substitute for conventional protein

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supplements, mainly in the feed industry (Chanda and Chakrabarti 1996). SCP production has been developed because microbes can be used to ferment a variety of agro-industrial wastes. The reuse of these waste materials can reduce the cost of SCP production significantly (Ravindra 2000). In recent years, several studies on SCP production from agro-industrial wastes such as pineapple cannery effluent (Nigam 1998), deproteinized whey concentrates (Schultz et al. 2006), rice polishings (Rajoka et al. 2004) and sugar cane bagasse hemicellulosic hydrolyzate (Nigam 2000) have been reported.

Among microorganisms used for the production of SCP, yeasts, especially *Saccharomyces* spp. and *Candida* spp. are generally regarded as safe (GRAS) and have been applied extensively to the reuse of a variety of waste materials. Moreover, yeasts are more convenient for use with cheap raw materials, can be harvested more easily than any other microorganism, and contain lower amounts of nucleic acids per cell than bacteria (Bekatorou et al. 2006).

Soy molasses is a by-product generated in the process of producing soy protein concentrate by precipitating soybean protein using ethanol aqueous solution. With the increasing demand for high quality plant proteins in the feed and food industry, the production of soy protein concentrates has increased by more than 10% annually for the past several years (Deak and Johnson 2006). Consequently, large quantities of soy molasses are generated annually. Soy molasses typically contains more than 50% soluble solids consisting essentially of about 60% carbohydrates, 10% nitrogenous substances, 20% lipids and 10% minerals (Kinney 2003). At present, soy molasses is applied mainly to the feed industry as a feed ingredient in mixed feeds. Moreover, soy molasses as a low-cost byproduct is a potentially fermentative feedstock because of its high fermentable sugar content. In recent years, a few studies

have reported the use of soy molasses as a fermentation medium for reducing the cost of some products such as sophorolipids by *Candida bombicola* (Solaiman et al. 2007), bio-ethanol by *Saccharomyces cerevisiae* (Siqueira et al. 2008), hydroxyalkanoates by *Pseudomonas corrugata* (Solaiman et al. 2006) and butanol by *Clostridium beijerinckii* BA101 (Qureshi et al. 2001).

To date, there have been no reports on using soy molasses as a fermentation substrate for SCP production. In this paper, we described SCP production by yeasts using soy molasses as a potential low-cost substrate.

Materials and methods

Organism and culture conditions

Geotrichum candidum CGMCC 2.498, *Candida tropicalis* CGMCC 2.587 and *Candida utilis* CGMCC 2.120 were obtained from China General Microbiological Culture Collection Center. *Geotrichum candidum* CGMCC 2.1035 and *C. utilis* CGMCC 2.1180 were kindly provided by Institute of Microbiology in Heilongjiang Province, China. *Candida utilis* A1, *Saccharomyces ellipsoideus* G1 and *S. cerevisiae* A1 were obtained from Food College of Heilongjiang Bayi Agricultural University, China. All these strains were stored in yeast peptone dextrose (YPD) agar medium containing 1.0% yeast extract, 2.0% glucose, 2.0% polypeptone and 1.5% agar and stored at 4°C. Before use, cultures were grown in YPD broth at 30°C with constant shaking (100 rpm) for 18 h.

Soy molasses

Soy molasses was kindly supplied by Heilongjiang Shuanghe Songnen Soybean Bioengineering Co., (Daqing, China). The concentrations of soluble solids and total sugar of soy molasses were 60.5% and 34.5%, respectively. The major sugars in soy molasses were sucrose (21.7%), stachyose (10.3%) and raffinose (2.1%).

Selecting yeast for SCP production from soy molasses

To adapt them for SCP production from soy molasses, yeasts were grown on soy molasses agar plates and in soy molasses broth.

Initially, eight yeast strains were screened for growth on soy molasses agar plates. Cultures were serially ten-fold diluted with sterile water and liquor samples (0.1 mL, 10^{-5} dilution, about 10^3 CFU/mL) were spread on the surface of soy molasses agar plates (30 g wet weight soy molasses per

100 mL medium). After incubation at 30°C for 3–4 days, the number of viable cells (CFU/mL) was measured and the growth status observed. Strains that could grow faster and form thicker colonies on soy molasses agar plates were selected.

The strains obtained by preliminary screening on soy molasses agar plates were cultured in YPD broth at 30°C with constant shaking (100 rpm) for 18 h. These cultures were inoculated into soy molasses broth (10 g wet weight of soy molasses per 100 mL medium) and cultivated with shaking at 160 rpm and 30°C for 24 h. The cells were harvested by centrifugation (4,000 rpm, 10 min), washed three times using sterile saline, and then dried to constant weight at 105°C. Finally, the optimal strain was determined after analyzing cell dry weight, protein content in dried cells and total protein.

Fermentation tests in triangular flasks

One loop of cells of the optimal strain was transferred to 50 mL malt extract medium (pH 5.0) in a 250-mL triangular flask and cultivated by shaking at 160 rpm and 30°C for 16 h. The initial biomass concentration of the fermentation medium was adjusted to approximately 1×10^8 CFU/mL. The effect of soy molasses concentration (1, 3, 5, 7, 9 and 11 g/100 mL medium containing 1, 3, 5, 7, 9 and 11 g wet weight soy molasses) on SCP production was conducted in 250-mL triangular flasks containing 50 mL medium and cultivated in a vibrating shaking incubator at 30°C (160 rpm) for 24 h. Effects of initial pH (4.0, 4.5, 5.0, 5.5, 6.0 and 6.5), nitrogen source (0.5 g/L yeast extract, peptone, sodium nitrate, ammonium sulfate and urea), nitrogen source concentration (0.2, 0.4, 0.6, 0.8 and 1.0 g/L), mineral salt (0.02 g/L CaCl$_2$, NaCl, MgSO$_4$·7H$_2$O, K$_2$HPO$_4$ and ZnSO$_4$·7H$_2$O) and mineral salt concentration (0.01, 0.02, 0.03, 0.04 and 0.05 g/L) on SCP production were tested in 250-mL triangular flasks containing 50 mL medium (7 g wet weight of soybean molasses per 100 mL medium) and cultivated as above. Soy molasses medium without supplementation of nitrogen source and mineral salt was used as a control. Finally, the strain was grown in soy molasses medium (soybean molasses concentration 7 g/100 mL and without supplementation of nitrogen source and mineral salt) at different temperatures (21, 24, 27, 30, 33 and 36°C) as above for 24 h.

Fermentation tests in 10-L bioreactor

Batch fermentations were performed in a 10-L bioreactor (GBJS-10, Zhenjiang East Biotech Equipment and Technology, Zhenjiang, China) containing 4 L optimized

fermentation medium (7 g wet weight of soy molasses per 100 mL medium, 0.2 g/L yeast extract, 0.02 g/L CaCl$_2$). Biomass concentration at the beginning of the fermentation was adjusted to approximately 1×10^8 CFU/mL. Agitation rate and airflow rate were set at 200 rpm and 3 L/min, respectively. Temperature and pH were maintained at 30°C and 5.5. Cell dry weight, total protein and total sugar were analyzed at intervals of 3 h over 30 h of fermentation.

Analytical methods

Cell dry weight of cultures was analyzed by centrifuging at 4,000 rpm for 15 min, washing the pellet three with distilled water and drying at 105°C to constant mass.

Protein content in dried cells was measured by the Kjeldahl method for nitrogen (Strickland and Parsons 1972). Total protein (g/L) was expressed as the amount of protein produced by *C. tropicalis* CGMCC 2.587 in 1 L medium.

Sucrose, raffinose and stachyose were analyzed using a PC-2025 HPLC system (SSI, LabAlliance, http://www.laballiance.com) consisting of a Series III pump (LabAlliance), a Thermo NH3 column (250×4.6 mm, 5 μm) and a RID-2001 detector (LabAlliance). The mobile phase was 75% acetonitrile aqueous solution and flow rate was 1.0 mL/min. Standards were 2.5 mg/mL sucrose (Sigma, St. Louis, MO), raffinose (Sigma) and stachyose (Sigma). Individual sugar concentrations were calculated by standard curves relating concentration to peak area. Total sugar was measured by the anthrone reagent method (Zill 1956).

The amino acid composition of the SCP was analyzed using automatic amino acid analyzer (Hitachi L-8800, Techcomp, Tokyo, Japan) according to the method described by Tong et al. (2009). Nucleic acid content of SCP was measured by colorimetric assays as described by Kochert (1978).

Statistical analysis

Each experiment was repeated three times. All data was analyzed using Microsoft Excel (Version 2003). Significance differences were analyzed using SAS 8.1 (SAS Institute, Cary, NC).

Results

Selecting the yeast for SCP production

Initially, eight strains were screened for growth on soy molasses agar plates. Among these strains, *G. candidum* CGMCC 2.498, *C. tropicalis* CGMCC 2.587, *C. utilis* CGMCC 2.120, *G. candidum* CGMCC 2.1035 and *C. utilis* CGMCC 2.1180 grew comparatively faster and formed bigger and thicker colonies on soy molasses agar plates after 24 h of cultivation. *Candida utilis* A1, *S. ellipsoideus* G1 and *S. cerevisiae* A1 grew comparatively more slowly and formed smaller and sparser colonies (data not shown).

Therefore, the former five strains were cultivated in soy molasses broth to compare their potential to support cell biomass and total protein yield. Among these five strains, cell dry weight and total protein yield of *C. tropicalis* CGMCC 2.587 were both highest, at 8.41 g/L and 4.47 g/L, respectively (Table 1). The protein content in dry cells of the strain was 53.13%. These results indicated that *C. tropicalis* CGMCC 2.587 was able to grow faster in soy molasses broth and convert the substances present in soy molasses to protein. Therefore, *C. tropicalis* CGMCC 2.587 was selected as the optimal strain for SCP production from soy molasses.

Effect of soy molasses concentration on SCP production

Total protein was increased from 2.61 to 4.70 g/L, and cell dry weight was increased from 5.43 to 8.82 g/L when the soy molasses concentration was increased from 1 to 7 g/100 mL (Table 2). But when the soy molasses concentration was increased further from 7 to 11 g/100 mL, total protein and cell dry weight were decreased to 3.95 and 7.44 g/L, respectively (Table 2). The results suggested that 7 g/100 mL of soy molasses was the most suitable concentration for SCP production by *C. tropicalis* CGMCC

Table 1 Cell dry weight, protein content in dried cells and total protein of five yeast strains grown on soybean molasses broth

Strain	Cell dry weight (g/L)	Protein content in dried cells (%)	Total protein (g/L)
Geotrichum candidum CGMCC 2.498	6.64±0.18	52.06±1.06	3.46±0.09
G. candidum CGMCC 2.1035	6.86 ±0.21	51.96±2.11	3.57±0.12
Candida tropicalis CGMCC 2.587	8.41±0.12	53.13±1.35	4.47±0.07
Candida utilis CGMCC 2.120	5.38±0.25	57.32±1.88	3.08±0.08
C. utilis CGMCC 2.1180	6.66±0.16	60.99±2.04	4.06±0.11

Table 2 Effect of soybean molasses concentration on single cell protein (SCP) production

Soybean molasses concentration (g/100 mL)	Cell dried weight (g/L)	Total protein (g/L)
1	5.43±0.10	2.61±0.16
3	6.48±0.21	3.22±0.12
5	6.98±0.14	3.71±0.09
7	8.82±0.20	4.70±0.15
9	8.24±0.19	4.38±0.11
11	7.44±0.24	3.95±0.13

2.587. Total sugar in the medium was decreased to 4.92 g/L at this concentration (data not shown).

Effect of initial pH on SCP production

The pH value of the fermentation process for producing SCP is normally in the range of pH 4.5–5.5 because of yeasts and filamentous fungi being acidophiles (Vicente et al. 1998). The effect on SCP production by *C. tropicalis* CGMCC 2.587 of initial pH in the range of 4.0–6.5 is shown in Table 3. When the initial pH value of the medium was adjusted to 5.5, total protein and cell dry weight attained maximum yields of 5.05 and 8.96 g/L after 24 h of fermentation (Table 3).

Effect of nitrogen source supplementation on SCP production

The results in Table 4 show that total protein increased from 4.63 to 5.24 g/L and cell dry weight increased from 8.73 to 9.36 g/L when 0.5 g/L yeast extract was supplemented in soy molasses medium, and inorganic nitrogen (0.5 g/L sodium nitrate, ammonium sulfate and urea) has no facilitating effect on SCP production (Table 4).

As shown in Fig. 1, total protein was increased from 4.61 to 5.34 g/L and cell dry weight was increased from 8.68 to 9.51 g/L, when soy molasses medium was supplemented with 0.2 g/L yeast extract. But when the supplemented amount of yeast extract was increased

Table 3 Effect of initial pH on SCP production

Initial pH	Cell dried weight (g/L)	Total protein (g/L)
4.0	6.31±0.12	3.16±0.08
4.5	6.95±0.19	3.61±0.11
5.0	8.01±0.21	4.26±0.14
5.5	8.96±0.18	5.05±0.12
6.0	8.44±0.21	4.49±0.15
6.5	7.41±0.17	3.93±0.14

Table 4 Effect on SCP production of supplementation with nitrogen source

Nitrogen source (0.5 g/L)	Cell dried weight (g/L)	Total protein (g/L)
Control	8.73±0.21	4.63±0.12
Yeast extract	9.36±0.23	5.24±0.10
Peptone	8.77±0.18	4.66±0.13
Sodium nitrite	8.50±0.16	4.32±0.15
Ammonium sulfate	8.32±0.22	4.23±0.12
Urea	8.26±0.20	4.05±0.16

from 0.2 to 1.0 g/L, total protein and cell dry weight were not notably increased ($P > 0.05$) (Fig. 1). Because the cost of commercial yeast extract is relatively high, the most suitable amount of yeast extract was deemed to be 0.2 g/L.

Effect on SCP production of inorganic salt supplementation

As shown in Table 5, total protein increased from 4.58 to 5.60 g/L and cell dry weight was increased from 8.62 to 9.95 g/L when $CaCl_2$ (0.05 g/L) was supplemented to soy molasses medium, whereas the addition of NaCl, $MgSO_4 \cdot 7H_2O$, K_2HPO_4 and $ZnSO_4 \cdot 7H_2O$ (0.05 g/L) had no notable effect ($P > 0.05$). These results indicate that $CaCl_2$ in the medium is important for the production of SCP by *C. tropicalis* CGMCC 2.587. The results of Iida et al. (1990) also showed that addition of Ca^{2+} obviously influenced the cell cycle of yeast and might regulate the level of cyclic adenosine monophosphate.

The results presented in Fig. 2 show that total protein and cell dry weight reached a maximum of 5.67 and
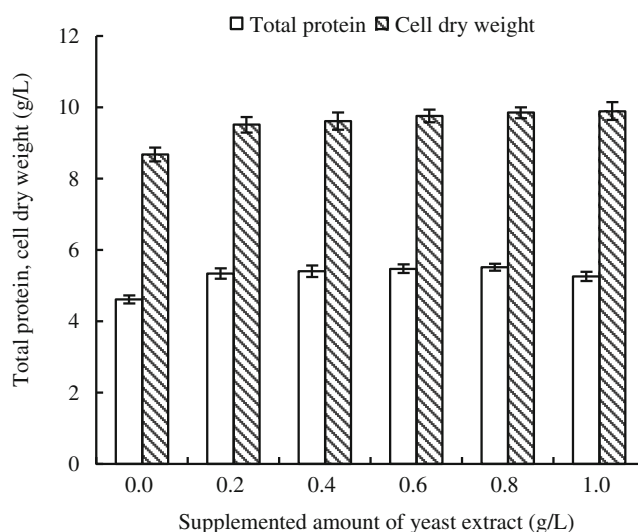

Fig. 1 Effect on single cell protein (SCP) production of supplementing with yeast extract
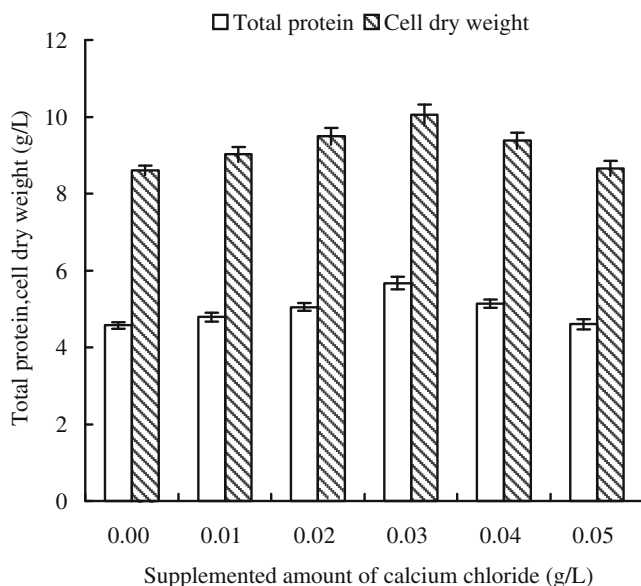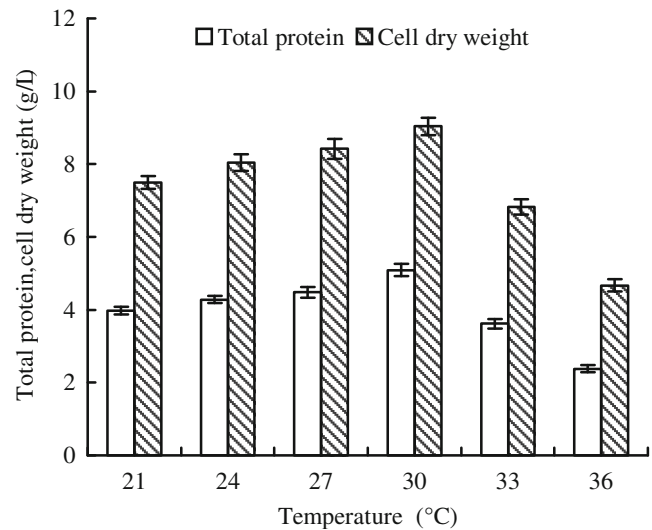
Table 5 Effect on SCP production of supplementation with inorganic salt

Inorganic salt (0.05 g/L)	Cell dried weight (g/L)	Total protein (g/L)
Control	8.62±0.18	4.58±0.10
CaCl$_2$	9.95±0.21	5.60±0.18
NaCl	8.48±0.19	4.51±0.14
MgSO$_4$·7H$_2$O	8.59±0.17	4.57±0.11
K$_2$HPO$_4$	8.94±0.23	4.76±0.13
ZnSO$_4$·7H$_2$O	8.37±0.21	4.45±0.14

10.06 g/L, respectively, when the supplemental amount of CaCl$_2$ was 0.03 g/L, and increased by more than 15% compared to those media not supplemented with CaCl$_2$. When the amount of CaCl$_2$ added to the medium was more than 0.03 g/L, total protein and cell dry weight began to decrease. Therefore, the suitable inorganic salt supplementation in fermentation medium was 0.03 g/L CaCl$_2$.

Effect of temperature on the production of SCP

Temperature is one of the main factors that can significantly influence cell growth and the production of metabolites in any bioprocess. The temperature range for SCP production is generally held to be 25–35°C (Ugalde and Castrillo 2002). The effect on SCP production from soy molasses by *C. tropicalis* CGMCC 2.587 of fermentation temperature in the range of 21–36°C is shown in Fig. 3; maximum yields of total protein and cell dry weight were obtained when the fermentation temperature was held at 30°C. Therefore,

Fig. 3 Effect of temperature on SCP production

the optimal fermented temperature for SCP production from soy molasses medium by *C. tropicalis* CGMCC 2.587 was 30°C.

Production of SCP in a 10-L bioreactor

In a triangular flask, the optimal medium for SCP production was 7 g/100 mL soy molasses, 0.2 g yeast extract and 0.03 g CaCl$_2$ added per liter medium (pH 5.5) at 30°C. Under these conditions, and a agitation rate of 200 rpm and airflow rate of 3 L/min, maximum yields of cell dry weight and total protein attained were 10.83 and 6.11 g/L after 30 h of fermentation in a 10-L bioreactor (Fig. 4). Protein content in dried cells produced by *C. tropicalis* CGMCC 2.587 was 56.42%.

Amino acid composition and nucleic acid content in SCP

Amino acid composition analysis showed that the protein produced by *C. tropicalis* CGMCC 2.587 from soy molasses contains high levels of the amino acids essential for animal feed (Table 6). Among the essential amino acids in the protein produced by *C. tropicalis* CGMCC 2.587, with the exception of cystine and isoleucine, the concentrations of lysine, threonine, valine, methionine, leucine and phenylalanine were higher than those of FAO standards (Table 6). With the exception of leucine, isoleucine and valine, the contents of essential amino acids in the protein produced by *C. tropicalis* CGMCC 2.587 were higher than those of soybean (Table 6).

In general, the nucleic acid content of SCP should be between 1% and 11% (Rajoka et al. 2004). In this study, the nucleic acid content of SCP produced by *C. tropicalis* CGMCC 2.587 was found to be 5.28%, which is
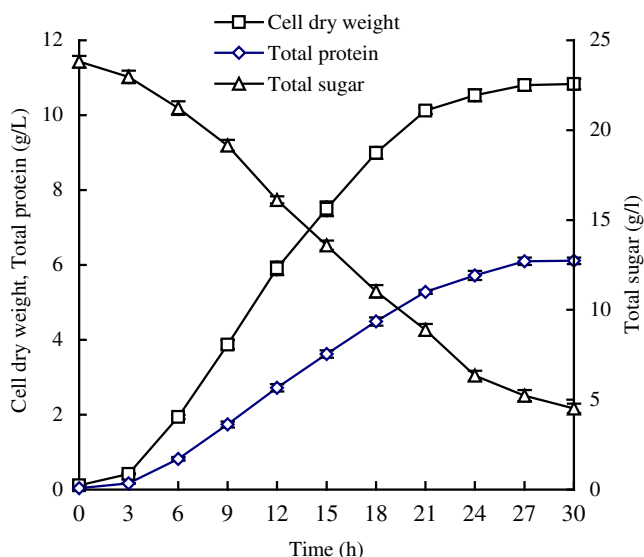

Fig. 2 Effect on SCP production of supplemented amount of calcium chloride

Fig. 4 Changes in cell dry weight, total protein and total sugar soybean molasses medium underoptimized conditions by *Candida tropicalis* CGMCC 2.587 during batch fermentation in a 10-L bioreactor

significantly lower than values reported by Nigam (1998, 2000) and Gao et al. (2007).

Consumption of sugar

The main sugars consumed by *C. tropicalis* CGMCC 2.587 were analyzed by HPLC after 30 h of fermentation in a 10-L bioreactor (Fig. 5). Finally, 98.2% of sucrose, 56.1% of raffinose and 46.9% of stachyose present in soy molasses medium were utilized by *C. tropicalis* CGMCC 2.587. After 30 h of fermentation, 80.3% of total sugars of soy molasses were consumed by *C. tropicalis* CGMCC 2.587,
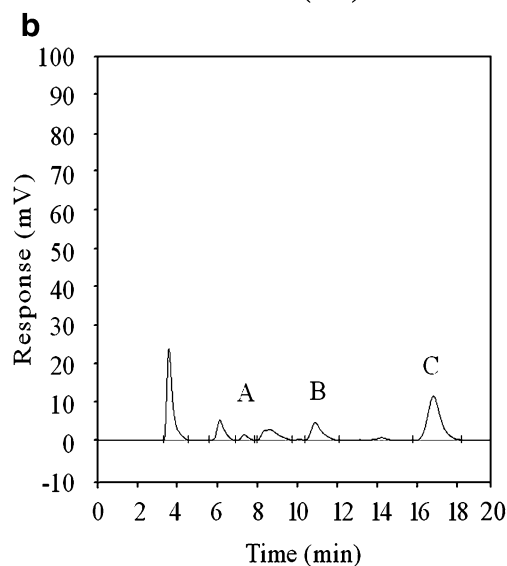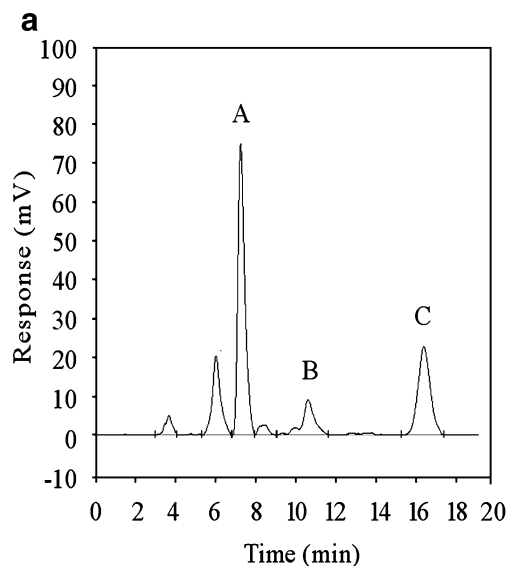

Fig. 5 HPLC analysis of the main sugars present in **a** unfermented and **b** fermented soy molasses medium. Sucrose (*A*), raffinose (*B*) and stachyose (*C*) were identified by comparing withstandards

and only 19.7% of residual sugars remained in the soy molasses medium.

Table 6 Amino acid composition of protein produced by *C. tropicalis* CGMCC 2.587 and compared with FAO and soybean

Amino acid	Concentration (% of total protein)		
	C. tropicalis CGMCC 2.587[a]	FAO[b]	Soybean[c]
Lysine	6.91	6.60	6.60
Threonine	4.35	2.80	4.30
Cystine	1.41	2.00	1.60
Valine	4.58	4.20	5.00
Methionine	2.27	2.20	1.30
Isoleucine	4.00	4.20	4.90
Leucine	6.24	4.80	8.00
Tryptophan	1.52	1.40	1.40
Phenylalanine	3.71	2.80	–

[a] This work

[b] Araujo and D'souza (1986)

[c] Lo and Moreau (1986)

Discussion

Candida species can grow in various by-products of the food industry and agriculture (Bekatorou et al. 2006). Several studies have reported the use of *Candida* yeasts to produce SCP using a variety of such by-products and wastes, such as salad oil manufacturing wastewater (Zheng et al. 2005a), lettuce brine (Suntornsuk 2000), molasses and sugar beet pulp (Nigam and Vogel 1991) and rice straw hydrolysate (Zheng et al. 2005b). From the results shown in Table 1, we also found that the three *Candida* strains used

have a higher protein content in dried cells than the two *Geotrichum* strains tested when cultivated in soy molasses medium.

It has long been known that the initial pH of the medium has a notable effect on the production of SCP (Ravindra 2000). The pH of fermentation processes for producing SCP is normally in the range of 4.5–5.5 because yeasts and filamentous fungi are generally acidophiles (Vicente et al. 1998); the results shown in Table 3 are in accordance with this rule. Yeasts are heterotrophic organisms and their growth requires a supply of organic carbon and a nitrogen source. The results presented here also indicate that addition of yeast extract to soy molasses medium was beneficial to the growth of the yeast strain and to conversion of protein in the cell, even though soy molasses already contains a considerable amount of protein. Solaiman et al. (2007) also reported that supplementation of yeast extract and urea can improve the production of sophorolipids from soy molasses by *C. bombicola* ATCC 22214, and that omission of these two nitrogen sources decreased product yield by about 30%. Iida et al. (1990) reported that addition of Ca^{2+} clearly influenced the yeast cell cycle, possibly via regulation of the level of cyclic adenosine monophosphate. The results in this study also indicated that $CaCl_2$ in the medium was important for the growth of *C. tropicalis* CGMCC 2.587 and production of SCP.

Under optimized conditions, maximum yields of cell dry weight and total protein reached 10.83 and 6.11 g/L after 30 h of fermentation in a 10-L bioreactor (Fig. 4). Protein content in dry cells produced by *C. tropicalis* CGMCC 2.587 was 56.41%. The cell mass obtained in this study was higher than those reported by Nigam (1998), Rajoka (2005), and Gao et al (2007). In this study, the nucleic acid content of SCP (5.28%) was significantly lower than that reported by Nigam (1998), Nigam (2000) and Gao et al. (2007). The protein produced by *C. tropicalis* CGMCC 2.587 contains all amino acids essential for animals. Analysis of the amino acid composition and nucleic acid content suggests that *C. tropicalis* CGMCC 2.587 is suitable for the production of high quality SCP using low-cost soy molasses as a substrate.

Stachyose, raffinose and sucrose are the main sugars present in soy molasses, and all have β-1,2 bonds that can be hydrolyzed by β-D-fructofuranoside fructohydrolase (E.C. 3.2.1.26). It is known that yeasts generally produce β-D-fructofuranoside fructohydrolase, which can cleave sucrose into glucose and fructose (Zech and Görisch 1995). Unlike sucrose, stachyose and raffinose contain α-1,6 bonds that can be hydrolyzed by α-galactosidase (E.C. 3.2.1.22)—which is probably not produced by *Saccharomyces cerevisiae*—resulting in a high concentration of residual sugars (48% of the soybean molasses' sugars) after ethanol fermentation (Siqueira et al. 2008). These results show that *C. tropicalis* CGMCC 2.587 might produce not only β-D-fructofuranoside fructohydrolase but also α-galactosidase, and therefore demonstrates good potential to treat soy molasses and produce SCP at a low cost.

The results of this study demonstrate that soy molasses has potential as a low-cost fermentation substrate for SCP production. The strain *Candida tropicalis* CGMCC 2.587 had a stronger ability to utilize the carbohydrates existing in soy molasses. SCP produced by *C. tropicalis* CGMCC 2.587 using soy molasses as a substrate had high protein quality and protein content, low nucleic acid content and was rich in amino acids essential for animals.

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