Bioresource Technology 124 (2012) 77–82

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

High level lipid production by a novel inulinase-producing yeast *Pichia guilliermondii* Pcla22

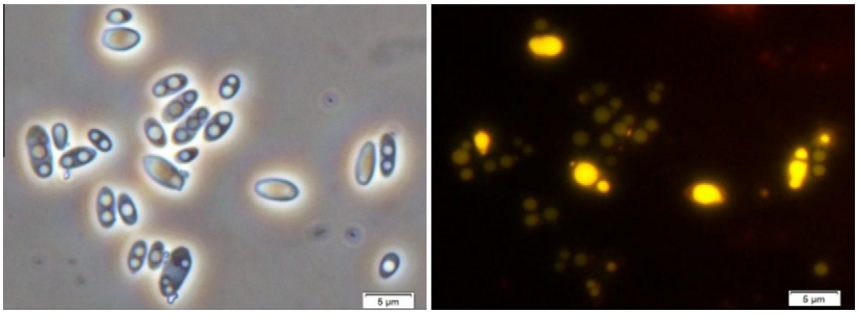
Guang-Yuan Wang [a,b], Zhe Chi [a], Bo Song [a], Zhi-Peng Wang [a], Zhen-Ming Chi [a,*]

[a] Unesco Chinese Center of Marine Biotechnology, Ocean University of China, Yushan Road, No. 5, Qingdao, China
[b] Shandong Provincial Key Laboratory of Applied Mycology, Qingdao Agricultural University, Changcheng Road, No. 700, Qingdao, China

HIGHLIGHTS

- The inulinase-producing yeast strain Pcla22 of *Pichia guilliermondii* was obtained.
- Lipid content in the yeast cells cultivated a flask reached 55.2%.
- 60.6% of lipid was obtained after the fed-batch fermentation.
- Many lipid bodies were produced in the yeast cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 June 2012
Received in revised form 3 August 2012
Accepted 4 August 2012
Available online 14 August 2012

Keywords:
P. guilliermondii
Single cell oil
Inulin
Inulinase
Biodiesel production

ABSTRACT

In this study, an inulinase-producing yeast strain Pcla22 of *Pichia guilliermondii* was identified. It was found that the yeast strain Pcla22 could produce higher amount of oil and more lipid bodies in its cells than any other yeast strains tested in this study. Under the optimal conditions, 60.6% (w/w) of lipid based on cell dry weight, 20.4 g/l of the dry cell mass, SCO produced per g of consumed sugar of 0.19 g/g and biomass produced per g of consumed sugar of 0.32 g/g were obtained in the culture of the yeast strain Pcla22 after 96 h of the fed-batch fermentation. Over 79.8% of the fatty acids from the yeast strain Pcla22 grown in the oil production medium containing inulin was $C_{16:0}$ and $C_{18:1}$, especially $C_{18:1}$ (57.9%). The biodiesel obtained from the produced lipid could be burnt well.

1. Introduction

In recent years, many researchers have paid much attention to single cell oil production by different microorganisms as the lipids extracted from the oleaginous microorganisms can be transformed into biodiesel by chemical ways or biological ways (Helwani et al., 2009). It has been well documented that biodiesel has many advantages over the conventional diesel because of its biodegradable, non-toxic, and essentially free of sulfur and aromatic components (Helwani et al., 2009). The yeast lipids were also used as substitutes of high added value exotic fats (e.g., cocoa butter) (Papanikolaou and Aggelis, 2011b). It has been known that many bacteria, filamentous fungi, yeasts and algae are oleaginous ones and can produce over 30% (w/w) lipid in their cells (Meng et al., 2009). However, it has been found that the oleaginous yeasts are the better ones because of rapid unicellular growth, high cell mass, high content of lipid, easily genetic modification, no endotoxin and easily large scale fermentation (Zhao et al., 2010a,b). To date, *Yarrowia lipolytica*, *Rhodosporidium toruloides*, *Lipomyces starkeyi*, *Trichosporon fermentans*, *Rhodotorula mucilaginosa*, *Trichosporon capitatum*, *Apiotrichum curvatum*, *Candida curvata*, and *Cryptococcus curvatus* have been

* Corresponding author. Address: Unesco Chinese Center of Marine Biotechnology, Ocean University of China, Yushan Road, No. 5, Qingdao 266003, China. Tel./fax: +86 532 82032266.
E-mail addresses: zhenming@sdu.edu.cn, chi@ouc.edu.cn (Z.-M. Chi).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.08.024

intensively used to accumulate lipid from different carbon sources (Papanikolaou and Aggelis, 2011a). However, all the oleaginous yeasts do not synthesize and secrete the enzymes which can catalyze hydrolysis of polymers such as cellulose, starch, inulin and so on. Therefore, it is very important to get the oleaginous yeasts which can produce a large amount of polymer-degrading enzymes in order to greatly reduce the cost of raw materials for SCO production.

In the past years, inulin and inulin-containing materials have been confirmed to be ones of the best substrates for bio-product production (Chi et al., 2011). Inulin has linear chains of β-(2,1)-linked fructose residues attached to a terminal sucrose residue and it is widely stored in some flowing plants such as Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium endivia*) (Chi et al., 2011). Inulin is considered as a relatively inexpensive and abundant substrate for production of rich fructose syrups. Exo-inulinases hydrolyze terminal, non-reducing 2,1-linked β-D-fructofuranose residues in inulin, releasing β-D-fructose and the enzymatic hydrolysis with exo-inulinases from microbial sources is the one of the best approaches to obtain fructose free of undesirable by-products. Recently, they also have been used as the substrates for SCO production by oleaginous yeasts (Zhao et al., 2010a, 2011). Similarly, inulin must be hydrolyzed into glucose and fructose by exoinulinase from inulinase-producing yeasts or engineered oleaginous yeasts before it is transformed into SCO (Zhao et al., 2010b, 2011). Therefore, it is very significant to find the native oleaginous yeasts which can synthesize and secrete inulinase. In the previous studies (Gong et al., 2007, 2008; Zhang et al., 2009, 2010), *P. guilliermondii* strain 1 was found to be able to produce high level of inulinase and the inulinase from this yeast has been purified and characterized and its gene has been cloned and expressed. In order to know if *P. guilliermondii* can produce high amount of lipid in its cells, different strains of this yeast isolated from different sources were screened and their lipid contents were determined. Finally, it was found that the inulinase-producing yeast *P. guilliermondii* Pcla22 had 49% (w/v) of total lipid in its cells. Therefore, in the present study, high level lipid production from inulin by the inulinase-producing yeast *P. guilliermondii* Pcla22 was conducted. Recently, the genome of *P. guilliermondii* has been sequenced and become publicly available (http://www.broad.mit.edu). So it will be very easy to genetically modify and metabolically engineer the yeast in order to further improve lipid production from inulin by the yeast in the future. Except inulin, sugars or sugar-enriched wastes or residues, polysaccharides, N-acetylglucosamine, hydrolysates of various products or by-products, vegetable oils, pure free fatty acids, FAMEs, fatty byproducts or wastes, n-alkanes, ethanol, glycerol, and organic acids also have been used as materials for lipid production (Papanikolaou and Aggelis, 2011b). So far, it has been little known about how the yeasts accumulate high level of lipids from such substrates (Papanikolaou and Aggelis, 2011b).

2. Methods

2.1. Yeast strains

Strain QMD, strain 25–23a, strain TJY22, mutant M-30 which produced high level of inulinase (Yu et al., 2009), of *P. guilliermondii* and strain Pcla22 from Marine Microorganisms Culture Collection of China (http://www.mccc.org.cn) were preserved at −80 °C in this laboratory. These yeast strains were used to screen the SCO producers in this study.

2.2. Media

The yeast strains were kept at 4 °C on YPD agar slant. The medium for screening the yeast strains containing higher lipid content was the medium which contained KH_2PO_4, 0.7%; Na_2HPO_4, 0.25%; $MgSO_4{\cdot}7H_2O$, 0.15%; $CaCl_2$, 0.015%; $FeCl_3{\cdot}7H_2O$, 0.015%; $ZnSO_4{\cdot}7H_2O$, 0.002%; $(NH_4)_2SO_4$, 0.05%; yeast extract, 0.05%; glucose, 2.0% and pH 6.0 (Papanikolaou and Aggelis, 2002). The medium used for oil production by the yeast strains contained 0.7% KH_2PO_4, 0.25% Na_2HPO_4; 0.15% $MgSO_4{\cdot}7H_2O$; 0.015% $CaCl_2$; 0.015% $FeCl_3{\cdot}7H_2O$, 0.002% $ZnSO_4{\cdot}7H_2O$, 0.05% $(NH_4)_2SO_4$, 0.05% yeast extract, 5.0% inulin or glucose, pH 6.0 (Papanikolaou and Aggelis, 2002).

2.3. Screening of the yeast strains with high oil content

All the cultures were incubated in the screening medium in an orbital shaker at a shaking speed of 160 rpm and incubation temperature 28 °C for 72 h. The cells in the culture were collected and washed three times by centrifugation at 5000*g* and 4 °C with sterile saline water. The washed cells were dried at 80 °C until their weight was constant. The total lipids in the cells (1.0 g) were extracted according to Folch et al. (1957). The extracted lipids were weighted and oil content per 100 g of cell dry weight was calculated. Finally, it was found that the yeast strain Pcla22 among 5 yeast strains contained the highest amount of total lipids and the yeast strain TJY22 contained the lowest amount of lipid. Therefore, the two yeast strains were used in the subsequent investigations.

2.4. Staining and observation of lipid particles in the yeast cells

The yeast strain Pcla22 and the yeast strain TJY22 were grown in the oil production medium and YPD medium at 28 °C for 72 h by shaking, respectively. The cells were harvested and washed by centrifugation at 4 °C and 5000*g* for 10 min. The washed cells were stained with Nile Red (GenMed Scientifics Inc. USA; 0.5 mg/l in DMSO) for 5 min at room temperature. After stained, cells were observed under blue light with Olympus U-LH100HG fluorescent microscope with 100× oil immersion objective. Images were recorded using the cellSens Standard software.

2.5. Identification of the yeast

Routine identification of the yeast strain Pcla22 was performed using the methods described by Kurtzman and Fell (2000).

2.6. DNA extraction and PCR

The total genomic DNA of the yeast strain Pcla22 was isolated and purified by using the methods as described by Sambrook et al. (1989). Amplification and sequencing of D1/D2 26S rDNA sequences from this yeast strain were performed according to the methods described by Chi et al. (2007).

2.7. Phylogenetic analysis and identification of the yeast

The sequence obtained above was aligned using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi, last accessed 2007.10.18). The sequences which shared over 98% similarity with currently available sequences were considered to be the same species and multiple alignments were performed using ClustalX 1.83 and phylogenetic tree was constructed using MEGA 4.0 (Tamura et al., 2007).

2.8. Cloning and sequencing of the INU1 gene encoding exo-inulinase

The total genomic DNA of the yeast strain Pcla22 was isolated and purified as described above. The primers for cloning of the *INU1* gene in the yeast strain Pcla22 were designed according to the *INU1* gene (the Accession number: EU195799) in *P. guilliermondii* strain 1 (Zhang et al., 2009). The *INU1* gene was cloned by PCR