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Single cell oil production from hydrolysate of cassava starch by marine-derived yeast *Rhodotorula mucilaginosa* TJY15a

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ABSTRACT

Rhodotorula mucilaginosa TJY15a which was isolated from surface of marine fish could accumulate a large amount of lipid from hydrolysate of cassava starch. The cells contained 47.9% (w/w) oil during batch cultivation, whereas 52.9% (w/w) of lipid was obtained during the fed-batch cultivation. At the end of the fed-batch cultivation, all the starch were converted into reducing sugar and only 0.34 g dm^{-3} of reducing sugar was left in the fermented medium. Therefore, the marine-derived *R. mucilaginosa* TJY15a was another candidate for single cell oil production. The fatty acids from *R. mucilaginosa* TJY15a were mainly composed of palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$) and linolenic acid ($C_{18:2}$), suggesting that the fatty acids could be used as feedstock for biodiesel production.

1. Introduction

In the past years, biodiesel, which is a renewable, biodegradable, and nontoxic fuel has received increasing attention because of the environmental pollution and energy crisis world wide. Biodiesel can be produced by transesterification of triacylglycerols from renewable biomasss (single cell oils), yielding monoalkyl esters of long-chain fatty acids with short-chain alcohols. It has been well documented that single cell oils are produced by some oleaginous microorganisms, such as yeast, fungi, bacteria, and microalgae. In general, yeast and molds can accumulate much more lipids than bacteria and microalgae [1]. It has been demonstrated that single cell oils can be transformed to FAME (fatty acid methyl esters) by means of both enzymatic and inorganic catalysis. It was also found that the single cell oil production by oleaginous yeasts has many advantages due to their fast growth rate, high oil content and the resemblance of their triacylglycerol fraction to plant oil. So far, *Cryptococcus albidus*, *Cryptococcus curvatus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan*, and *Yarrowia lipolytica*, have been intensively used to accumulate oils from glucose, xylose, arabinose, mannose, glycerol, and other agricultural and industrial residues and different yeast species can accumulate different amount of oils in their cells [1–3].

At present the high production cost of biodiesel is a major barrier to its commercialization. The high cost of biodiesel production is partially associated with the cost of raw material, making it a less competitive fuel. Therefore, using a low cost raw material is crucial in reducing the cost of biodiesel production. In order to reduce the cost of the single cell oil production by yeasts, other carbon sources instead of glucose should be used.

Cassava (*Manihot esculenta*) is a root crop of tropical American origin and is the fourth most important staple

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crop in the tropics. It is also cultivated in many provinces in the south of China and cassava starch is produced on large scale in different regions of China. The cassava plant is extremely robust, is resistant to disease and drought, and can grow in relatively low-quality soils. In developing countries, it is well regarded that the grains cannot be used as the raw materials for biofuel production. Therefore, we think that cassava starch may be a good starchy material for biofuel production in China. Cassava starch is composed of unbranched amylose (20 ± 5%) and branched amylopectin (80 ± 5%), both of which can be hydrolyzed enzymatically (either with pure enzymes or amylase-producing microorganisms) to release their constituent glucose and maltooligosaccharides [4]. Because of high starch debranching activity, amylases produced by *Saccharomycopsis fibuligera* have extensively been used to hydrolyze cassava starch [5].

In recent years, it has been found that the marine environments are also rich in yeast resources and over 1200 strains belonging to different yeast species have been obtained in this laboratory [6] (http://www.mccc.org.cn, last accessed 2008.12.30). In this study, over 100 different yeast strains isolated from the marine fish *Synechogobius hasta* were screened for single cell oil production. It was found that *Rhodotorula mucilaginosa* TJY15a, one of the collection cultures of marine yeasts, could accumulate more oils from glucose than other yeasts tested in this study. Then, it was used to produce single cell oil from hydrolysate of cassava starch. This is the first report that *R. mucilaginosa* can accumulate high oil content in its cells.

2. Methods

2.1. Yeast strains

The single cell oil producer used in this study was *R. mucilaginosa* TJY15a which was isolated from the marine fish *S. hasta* collected at Bohai Sea (Longitude 117°2′ and Latitude 39°13′). The amylase producer was *S. fibuligera* IFO 0111 [4].

2.2. Media

The marine yeast strains were kept at 4 °C on YPD agar slant [7]. 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, 6.0% and pH 6.0. 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% $(NH_4)_2SO_4$, 0.33% yeast extract, 2.0% cassava starch or glucose, pH 6.0. The amylase production was carried out using the liquid medium which contained 1.0% cassava starch, 0.2% yeast extract, 0.06% $MgCl_2$, 0.0001% $MnSO_4 \cdot 7H_2O$, 0.01% $CaCl_2.2H_2O$, 0.2% $(NH_4)_2SO_4$, 0.2%KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ [8].

2.3. Sampling

The marine fish *S. hasta* was collected at Bohai Sea (Longitude 117°2′ and Latitude 39°13′) on November 27, 2007. After the fish was disinfected using 70% ethanol, the skin, gill and contents of its gut were used for the yeast isolation as described below.

2.4. Yeast isolation

Yeast isolation was carried out according to the methods [9]. Over 100 yeast strains were obtained in this study.

2.5. Screening of the marine-derived yeast strains with high oil content

All the cultures were incubated 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. [10]. The extracted lipids were weighted and oil content per 100 g of cell dry weight was calculated. Finally, it was found that strain TJY15a among 100 yeast strains contained the highest amount of total lipids. Therefore, the yeast strain TJY15a was used in the subsequent investigations.

2.6. Identification of the yeast

Routine identification of the yeast strain TJY15a was performed using the methods described by Kurtzman and Fell [11].

2.7. DNA extraction and PCR

The total genomic DNA of the yeast strain TJY15a was isolated and purified by using the methods as described by Sambrook et al. [12]. Amplification and sequencing of D1/D2 26S rDNA sequences from the yeast were performed according to the methods described by Chi et al. [7].

2.8. 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 are considered to be the same species and multiple alignments was performed by using ClustalX 1.83 and phylogenetic tree was constructed by MEGA 4.0 [13].

2.9. Preparation of amylase

One loop of the cells of the yeast strain *S. fibuligera* IFO 0111 was transferred to 50.0 ml of YPD medium in 250 ml flask and aerobically cultivated for 24 h. One milliliter of the cell culture ($OD_{600nm} = 30.0$) was transferred to the amylase production medium [8] and grown aerobically at 28 °C and 160 rpm for two days. The culture was centrifuged at 8000 × *g* and 4 °C for 10 min and the supernatant obtained was used the crude amylase preparation.