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Short communication

Microbial lipid production from pectin-derived carbohydrates by oleaginous yeasts

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ABSTRACT

Pectin-rich biomass is relatively less studied as feedstock for the production of biofuel and chemicals, partially because the major compositional carbohydrates galacturonate and arabinose are generally disfavored substrates for microorganisms. To explore the capability of microbial lipid production from pectic biomass, 10 oleaginous yeasts were evaluated by using galacturonate as sole carbon sources. Three strains belonging to *Trichosporon cutaneum*, *Trichosporon fermentans* and *Cryptococcus curvatus* were found to effectively convert galacturonate into lipid with lipid yields up to 0.12 g/g. Slightly higher lipid yields were also obtained on beet pulp hydrolysates, and cellular lipid contents were higher than 40.0% except for the *T. fermentans* strain. All of these lipid samples had long chain fatty acid compositional profiles similar to those of vegetable oils. The results demonstrate the potential of lipid production from pectic biomass, which is of great significance in terms of integrating wastes management and biofuel production.

1. Introduction

Biodiesel has been implemented as a major biofuel product worldwide in recent years in order to address several concerns related to overconsumption of fossil fuels. This has resulted in higher demands for vegetable oils and fats because biodiesel is produced from those commodity products. However, vegetable oils are limited resources due to their tight nexus with food industry and arable lands. Microbial lipids have been considered as an alternative feedstock for biodiesel production [1,2]. Oleaginous yeasts have some advantageous features for lipid production, such as fast growth rate, high lipid content, free of endotoxins, and broad feedstock spectrum [3]. Although microbial lipid production has been extensively investigated using glucose and starchy materials as the feedstock [4,5], the costs remain prohibitively high for commercialization. To reduce substrate costs, various raw materials such as molasses, industrial wastewater, crude glycerol and lignocellulosic biomass have been evaluated for microbial lipid production by different yeast species [6–8].

Pectin is a complex polysaccharide mainly composed of a backbone of covalently linked galacturonate with side chains containing various sugars such as arabinose, xylose and galactose [9]. Galacturonate comprises approximately 70% of pectin [10,11]. Large amounts of pectin-rich biomass such as sugar beet pulp, apple pomace and citrus peel are generated as wastes from industrial processing of fruits and vegetables [12]. Pectic biomass also contains cellulose and hemicelluloses, but very little lignin [13–15]. For sugar beet pulp, galacturonate, arabinose and glucose together take more than 60% (w/w) of its compositional carbohydrates. In another aspect, pectic wastes usually have gone through various processes such that no additional steps are required for particle size reduction, pretreatment or inhibitory compounds removal. Together, pectin-rich biomass is therefore an appealing resource for biological conversion compared to lignocelluloses. Some attempts have already been documented to produce ethanol from pectic wastes [12,16–18]. However, the yeast *Saccharomyces cerevisiae* is not an ideal producer because both galacturonate and arabinose are not fermentable. Although *Escherichia coli* can be used for ethanol production from these sugars, it tends to generate noticeable amounts of by-products such as acetate and succinate, leading to low ethanol yields. Therefore, more efficient routes remain to be developed for bioconversion of pectic wastes.

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In an early study, the oleaginous molds *Mortierella isabellina* ATHUM 2935 was tested for lipid production directly from apple pectin, and cellular lipid content and lipid yield reached 24% and 0.11 g/g [19]. In this work, we screened 10 oleaginous yeasts and identified 3 strains for lipid production from galacturonate as well as beet pulp hydrolysates. Higher lipid yields were obtained and lipid samples were found with similar fatty acid compositional profiles to those of commercial vegetable oils. Our results demonstrate new opportunity for bioconversion of pectic biomass into lipids, which is of great significance in terms of integrating wastes management and biofuel production.

2. Materials and methods

2.1. Strains and media

Ten oleaginous yeast strains were used in this work, including *Lipomyces starkeyi* AS 2.1560, *Yarrowia lipolytica* AS 2.1398, *Trichosporon cutaneum* AS 2.571, *Rhodotorula glutinis* AS 2.107, *Rhodotorula mucilaginosa* AS 2.1515 and *Rhodotorula minuta* AS 2.277 from the China General Microbiological Culture Collection Center, *Cryptococcus curvatus* ATCC 20509 and *Rhodosporidium toruloides* ATCC 10788 from the American Type Culture Collection, *Trichosporon fermentans* CICC 1368 from China Center of Industrial Culture Collection, *Rhodosporidium toruloides* Y4 from this lab [5]. These strains were all stored at 4 °C and propagated every two weeks on yeast peptone dextrose (YEPD) agar slants (glucose 20 g/L, yeast extract 10 g/L, peptone 10 g/L, agar 15 g/L, pH 6.0). Galacturonic acid was purchased from Fluka. Pectin from orange peels was obtained from J&K Chemicals (Cat. No. 254368). Dry sugar beet pulp was obtained from a local sugar plant in Inner Mongolia Autonomous Region, China. Analysis according to the NREL/TP-510-42618 protocol showed that the beet pulp sample contained major carbohydrate components of 23.4% arabinan, 28.3% galactan, 26.7% glucan and 4.6% xylan. Pectinase from *Aspergillus niger* was purchased from TCI (Cat. No. P0026). Cellulase from *Trichoderma reesei* was purchased from Zesheng Ltd. (Shandong, China). β-Glucosidase from *A. niger* was purchased from Sigma (St. Louis, USA). All other chemicals and reagents were bought locally and were of analytical reagent grade.

Assimilation medium contained (g/L): Galacturonic acid 20, $(NH_4)_2SO_4$ 5, KH_2PO_4 1, $MgSO_4{\cdot}7H_2O$ 0.5, agar 15. Galacturonic acid was replaced by glucose in control experiments, and the negative control was without carbon source. The lipid production medium contained (g/L): $(NH_4)_2SO_4$ 0.2, yeast extract 0.5, KH_2PO_4 2.7, $Na_2HPO_4{\cdot}12H_2O$ 2.4, $MgSO_4{\cdot}7H_2O$ 0.2, EDTA 0.1, and galacturonic acid (20) or sugar mixtures of galacturonic acid (20), arabinose (20) and glucose (20). Initial pH for all media was 6.0. Galacturonic acid, arabinose and glucose solutions were sterilized by filtration through 0.2 μm filter before added. Other components were sterilized by autoclaving at 121 °C for 20 min.

2.2. Pectin and sugar beet pulp hydrolysates preparation

The enzymatic hydrolysis of pectin at 2% (w/v) solid loading in deionized water was conducted at 50 °C, pH 4.0 for 90 min with pectinase loading of 20 mg/g. Dry beet pulp was milled and passed through 1-mm sized screen. The beet pulp was loaded at 10% (w/v) solid loading in 0.2 M phosphate buffer (pH 5.2) and hydrolyzed at 50 °C for 72 h in the presence of 20 FPU/g cellulose, 40 CBU/g β-glucosidase and 12 mg/g pectinase. The components of the lipid production medium were added at the appropriate concentration and final pH was adjusted to 6.0 before sterilization by filtration through 0.2 μm filter.

2.3. Carbohydrate assimilation test

Yeast cells were screened using culture plates in an incubator at 28 °C for 3 d. Pre-cultures were grown in YEPD liquid medium at 30 °C, 200 rpm for 24 h. Exact 5 mL of pre-cultures were inoculated in 45 mL of assimilation liquid medium or the control medium, and the cultures were held at 30 °C, 200 rpm for 48 h.

2.4. Lipid production

The cultures were initiated upon 45 mL of the lipid production medium inoculated with 5 mL of the pre-cultures. Experiments were done at 30 °C in 250-mL Erlenmeyer flasks on a rotary shaker of 200 rpm. All experiments were done in triplicates and results were averaged.

2.5. Analytical methods

The optical density of the culture was determined at 600 nm (OD_{600nm}) with an appropriate dilution with a V530 UV/vis spectrophotometer (JASCO, Japan). The pH of the culture was monitored with a pH/Ion 510 acidometer (Eutech Instruments, Singapore). Glucose was measured using an SBA-40D glucose analyzer (Shandong Academy of Sciences, Jinan, China). Galacturonate was determined according to a published procedure [20]. Total reducing sugars (TRS) were quantified according to the dinitrosalicylate (DNS) method [21]. Sugar mixtures were analyzed by ion chromatography (IC) on the Dionex ICS2500 system (Dionex Co., Sunnyvale, USA) with an analytical CarboPac PA10 column and an ED50 type electrochemical detector.

Cell mass was harvested by centrifugation, washed twice with distilled water, and determined gravimetrically after drying in an oven at 105 °C to a constant weight.

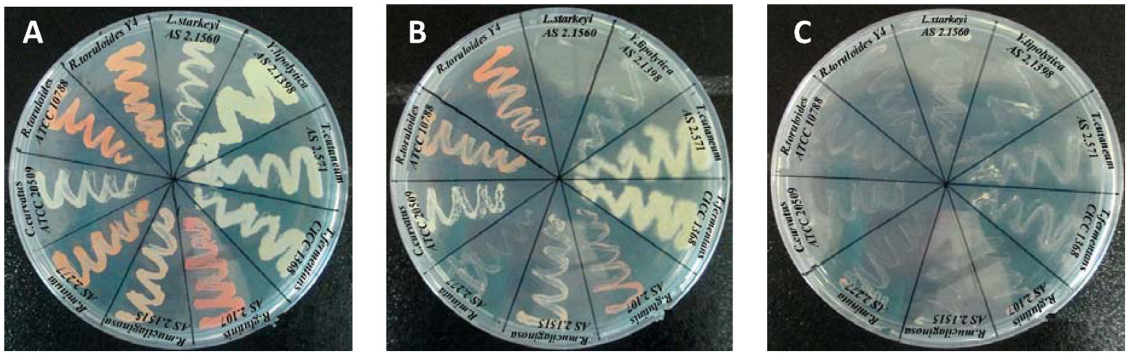

Fig. 1. Results of oleaginous yeast cell growth on agar plate using glucose (A) and galacturonate (B) as the sole carbon source and agar plate without carbon source (C).