Investigation of Liquid Culture Conditions for Triacylglycerol Production from Waste Peach by Lipomyces Wild-type Strain

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Abstract: In this study, waste peach (WP) liquid culture conditions for the maintenance of high triacylglycerol (TG)-accumulation ability in Lipomyces wild-type strain, obtained from WP plate medium were investigated. As the concentration of WP juice was high, the medium viscosity became high, and TG accumulation ability was suppressed. In a 5-L jar fermenter, the negative influence of viscosity on TG-accumulation ability was significantly improved by an agitation speed of 150 rpm (0.4vvm). Where a bench scale pilot plant (90-L jar fermenter) was operated at 40 rpm, TG-accumulation ability reached 6.8 mg/10⁶ cells. This ability was 85% of that obtained with WP plate medium.

Key words: yeast Lipomyces wild-type strain, triacylglycerol-accumulation ability, triacylglycerol production, waste peach juice, culture conditions

1 INTRODUCTION

Using biofuels, made from renewable resources, is an effective method to slow down global warming. In Japan, the self-sufficiency rate of vegetable oil is 3% on a calorie basis¹, making it unsuitable for biodiesel production. Lipids produced by microorganisms are an attractive source of biofuel. Moreover, microorganisms can be cultured at controlled conditions with fermenters. Multiple waste products have been utilized as substrates for lipid production for biofuels; industrial glycerol by Yarrowia lipolytica², food waste by Cryptococcus curvatus, Yarrowia lipolytica, Lipomyces starkeyi, Rhodosporidium toruloides, and Rhodotorula glutinis³, and corn starch waste water by Rhodotorula glutinis⁴ have already been reported.

In Minami-Alps city (Japan), peaches that cannot be shipped to the market are mainly abandoned, reaching 600 tons in a year. The yeast Lipomyces has the property to accumulate triacylglycerol (TG) in intracellular fat globules⁵, and Lipomyces wild-strain no. 347 shows high TG-accumulation ability (TG mg/10⁶ cells) in waste peach (WP) plate medium. The strain with high TG-accumulation ability has high TG conversion ratio to sugar consumed, and intracellular TG is easily leaked upon weak physical disruption. The TG produced using this yeast and WP, can be utilized as fuel for agricultural equipment engines and greenhouse heaters, thus reducing the cost of agriculture along with contributing to reduction in global warming. However, practical production of biodiesel would require large amount of TG, which may be obtained from liquid culture in large scale.

The aim of this study was to investigate the culture conditions in WP liquid medium for maintaining the property of high TG-accumulation in WP plate medium of strain no. 347; the amount of TG production was also examined. Supply of nutrients to WP liquid medium, suitable WP juice concentration, and the best agitation speed were investigated for maintenance of high TG-accumulation ability of strain no. 347. TG-accumulation ability and amount of TG production in strain no. 347, under optimized culture con-
ditions, were investigated using a bench scale pilot plant (90-L jar fermenter) containing WP liquid medium.

2 EXPERIMENTAL

2.1 Obtaining waste peach juice, and its properties

Peaches that cannot be shipped to the market and are hence abandoned, generate approximately 600 tons of waste per year in Minami-Alps city, Japan. The peaches used in this research were harvested during 2013–2014 (in July and August, each year). The waste peaches were crushed using a 5SW type crusher (TANINAKA O&K Co., Ltd., Osaka, Japan) and squeezed using a HC-JH (manual type) squeezer (SUN FOOD MACHINERY Co., Ltd., Tokyo, Japan). The pH value of the WP juice was adjusted to 2.2 with H2SO4 to inhibit growth of contaminating bacteria. The WP juice was frozen subsequently for quality retention.

On an average, 100 g of edible peach is composed of 88.7 g of water, 9–12 g of sugar (measured by a refraction meter), 1.3 g of dietary fibers, 0.6 g of protein, 0.4 g of ash, 0.1 g of lipid, 210.3 mg of minerals, and 9.5 mg of vitamins. Peach juice contains approximately 80% sucrose, 10% fructose, and 10% glucose.

2.2 Strain

Among the 428 strains that were screened, Lipomyces wild-type strain no. 347 (Lipomyces starkeyi NBRC 112453) was selected for its high TG-accumulation ability on WP plate medium.

2.3 Culture medium

The medium used for pre-culture consisted of 30 g glucose, 0.35 g (NH4)2SO4, 1.0 g KH2PO4, 0.5 g MgSO4·7H2O, 0.1 g NaCl, 0.1 g CaCl2·2H2O, 500 µg H3BO3, 40 µg CuSO4·5H2O, 100 µg KI, 200 µg FeCl3·6H2O, 400 µg MnSO4·4H2O, 400 µg ZnSO4·7H2O, 200 µg Na2MoO4·2H2O, and 2 µg biotin in 1 L of distilled water. A test tube (18 × 180 mm) containing 5 mL of distilled water. A test tube (18 × 180 mm) containing 5 mL of distilled water was transferred to a 5-L jar fermenter (B. E. MARUBISHI, MDL-8C and AIBLE, BMS-05NP3, DPC-3A) and was autoclaved at 121°C for 15 min. The adjusted medium (2.5 L) was transferred to a 5-L jar fermenter (B. E. MARUBISHI, MDL-8C and AIBLE, BMS-05NP3, DPC-3A) and was autoclaved at 121°C for 15 min. In case of using a bench scale pilot plant (90-L jar fermenter) (Biott, AIBLE, DPC-3A), the amount of adjusted medium was 45 L, and it was sterilized with steam at 121°C for 20 min.

2.4 Culture conditions

The starting cell number in each culture container was about 1 × 10⁶ cells/mL. The cell number was measured using a counting chamber (Thoma deep 0.1 mm; Erma). The test tubes and flasks used for culturing were shaken at 120 strokes/min with a reciprocal shaker at 28°C. Jar fermenter culture was performed with an aeration of 1.0 L/min (0.4 vvm) at 28°C. The agitation speed has been described in the Results and Discussion section.

2.5 Analytical method for triacylglycerol estimation

One milliliter of five-fold diluted culture broth was transferred into a test tube (18 × 110 mm) containing 2.5 g of glass beads (ϕ, 1.00–1.05 mm), and the test tube was shaken at 2,500 rpm for 60 min with a mixer (Model CM-1000; EYELA). Twenty microliters of the homogenate was mixed with three milliliters of Cleantech TG assay kit reagents (Triglyceride E-test Wako; Wako Pure Chemical Industries, Ltd.); this mixture was incubated at 37°C for 10 min. After centrifugation at 2,500 x g for 5 min, the supernatants were filtered using a 0.45-µm membrane filter (DISMIC-25CS; ADVANTEC). The absorbance of the filtrates was then measured at 600 nm.

2.6 Measurement of viscosity in medium

Culture medium was centrifuged at 2,500 x g for 5 min. Viscosity of the supernatant was measured at 28°C with the Ostwald viscometer (Relative Viscometer, SIBATA SCIENTIFIC TECHNOLOGY, Ltd.).

2.7 Definition of the TG production and TG-accumulation ability

TG production was defined as the amount of TG produced by the cells in the culture medium and calculated as: TG production (g/L) = TG (g)/culture medium (L).

TG-accumulation ability is the measure of the intracellular TG levels and was calculated as follows: TG (mg)/10⁶ cells = (TG [mg] per mL of culture medium)/(cell number per mL of culture medium) × 10⁶.

3 RESULTS AND DISCUSSION

3.1 Effect of nutrient supply to WP liquid medium on TG-accumulation ability, TG production, and total cell number

When strain no. 347 was cultured in the WP liquid medium, TG-accumulation ability was lower than on the WP plate medium. For obtaining high TG-accumulation ability in the WP liquid medium, nutrient supply was examined.

The YM medium, which is nutrient-rich, is used for yeast
3.2 Influence of WP juice concentration on TG-accumulation ability, TG production, and medium viscosity

For showing high TG-accumulation ability and obtaining large amount of TG production, the optimal WP juice concentration was explored. The results are shown in Fig. 2.

At 36% (4% sugar) and 45% (5% sugar) of WP juice concentration, high TG-accumulation ability was induced; at 45% and 55% (6% sugar), high production of TG was observed. Therefore, the optimal WP juice concentration for TG-accumulation ability and high TG production was determined to be 45%. However, the TG-accumulation ability, obtained in this experiment, was remarkably low compared to that in WP plate medium.

As the concentration of WP juice increased, viscosity of the medium increased, and TG-accumulation ability and TG production decreased (as shown in Fig. 2). The increase in viscosity was probably caused by the polysaccharide biosynthesized with strain no. 347.

Increasing viscosity has been reported to inhibit mass transfer and suppress metabolite production\(^9\). Thus, the depressed TG-accumulation ability and TG production in strain no. 347 with increased WP juice concentration was suggested to be caused by inhibition of mass transfer by viscosity.

3.3 Effect of agitation speed in jar fermenter on TG-accumulation ability and TG production

Agitation speed was investigated using a 5-L jar fermenter for improving mass transfer, since viscosity influenced mass transfer, and that in turn influenced TG-accumulation ability in strain no. 347. The results are shown in Fig. 3.

TG-accumulation ability was the highest at 150 rpm (0.4 vvm), which was about 94% of that obtained with WP plate medium. At 150 rpm, TG production was also high.
Several previous studies had reported the influence of increased viscosity on oxygen supply. The viscosity of WP liquid medium depends on oxygen supply and TG-accumulation ability of strain no. 347 also seems to be affected.

### 3.4 TG-accumulation ability and TG production in a bench scale pilot plant (90-L jar fermenter)

Using a bench scale pilot plant (90-L jar fermenter) containing WP liquid medium, maintenance of high TG-accumulation ability in strain no. 347, under optimized culture conditions, was investigated and the amount of TG production was measured. Results are shown in Fig. 4, and microphotograph of strain no. 347 cultured at 184 h is shown in Fig. 5.

The cell number increased until 64 h, after which cell proliferation became stationary. However, TG-accumulation ability increased from 40 h to 184 h, and amount of TG production increased similarly. These results suggest that the amount of TG production (g/L) was caused by the increase in TG-accumulation ability (mg/10^6 cells).

TG-accumulation ability and TG production were 6.8 mg/10^6 cells and 3.8 g/L at 184 h of culture, large fat globules observed as shown in Fig. 5. This TG-accumulation ability was 85% of that obtained with WP plate medium.

### 4 CONCLUSION

Optimal culture conditions, for the maintenance of high TG-accumulation ability of strain no. 347 in WP liquid
medium, were examined. Supplementation of WP liquid medium with nutrients increased cell proliferation, but suppressed TG-accumulation ability. An increase of WP juice concentration influenced viscosity of the culture medium, and inhibition of mass transfer by viscosity suppressed TG-accumulation ability and TG production in strain no. 347. For improving mass transfer, the effect of agitation speed in jar fermenter on TG-accumulation ability and TG production was investigated. Optimal agitation speed was 150 rpm with an aeration of 0.4vvm. Using a bench scale pilot plant (90-L jar fermenter) containing WP liquid medium, under optimized culture conditions, TG-accumulation ability and TG production in strain no. 347 were investigated. Approximately 6.8 mg/10^8cells with TG-accumulation ability was observed, which was 85% of that obtained when the strain was screened in plate culture. TG production was 3.8 g/L. Based on these results, strain no. 347 was found to efficiently biosynthesis TG in a large amount of WP liquid medium.

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