Selection of Oleaginous Yeasts and their Use for Lipid Production from Oil Palm Sap

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ABSTRACT

This study aimed to use oil palm sap squeezed from fresh oil palm trunk as an alternative carbon source for lipid production by oleaginous yeasts. Six oleaginous yeasts including *Trichosporonoides spathulata* JU4-57, *Kluyveromyces marxianus* X32, *Candida tropicalis* X37, *Rhodotorula mucilaginosa* G43, *Yarrowia lipolytica* TISTR 5151 and *Yarrowia lipolytica* TISTR 5054 were screened for their ability to grow and produce lipid in oil palm sap containing 20 g/L sugar with and without ammonium sulfate addition. Among six strains tested, *C. tropicalis* X37 and *R. mucilaginosa* G43 could grow well either with or without addition of ammonium sulfate. The maximum biomass and lipid obtained in the sap without addition of ammonium sulfate, were 11.05 g/L and 0.96 g/L for *C. tropicalis* X37 and 9.75 g/L and 1.0 g/L for *R. mucilaginosa* G43. With increasing sugar concentration up to 40 g/L, the lipid production by both strains were increased up to 1.35 g/L and 1.40 g/L, respectively. Fed-batch fermentation in which 20 g/L glucose was added at 36 h, 48 h and 60 h to maintain the C/N ratio at high level, could further increase the lipid production by both strains up to 2.85 g/L and 2.10 g/L, respectively. This study has shown that oil palm sap can be used for lipid production.

Keywords: Microbial oil, Oil palm sap, Oleaginous Yeast

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Introduction

Nowadays, there has been an increasing interest in looking for new oil sources for biodiesel production. Among them, microbial lipids produced by the so-called oleaginous microorganisms involving bacteria, yeasts, moulds, and microalgae are now considered as promising candidates because of their similar fatty acid composition to that of plant oils (Huang *et al*., 2009). Recently, various kind of low-cost substrates have been used to cultivate the oleaginous micro-organisms in order to reduce the production cost of lipid. Lignocellulosic biomass is one of potential substrates for lipid production due to its great availability in nature and renewable feature. Some lignocellulosic wastes such as rice straw, bagasse and corncob hydrolysate have been used for lipid production (Hadar, 2013). This not only reduce the production costs of lipid but also solve an environmental problem.

Oil palm (Elaeis guineensis) is one of the most important oil crops in Thailand. The area of oil palm plantations in Thailand in 2010 was 624,000 ha disseminating over 25 provinces of the country, mainly in the South (Department of Agriculture Extension, Bangkok, Thailand, 2010). On the whole, the palms bear oil-containing fruits after 3 years being planted and need to be replanted at intervals of 20–25 years to maintain oil productivity. In Thailand, most of the old oil palm trees, around 81 million cubic meters per year, are cut down and thrown out or burnt at the plantation site. However, attempts have also been made to use the old oil palm trees for different purposes depending on the tree parts. Oil palm trunk is one of the parts of the oil palm trees which have usually been used to make plywood in the logging industry. However, the core of the trunk is essentially soft and contains up to 80% of sap, which makes it not apposite source for making plywood. After the sap is squeezed from the trunk, the fiber wastes of the trunk left after this process are usually used as compost (Kosugi *et al*., 2010).

Objective of the study

The objective of this study was to produce lipids from oil palm trunk by using oleaginous yeasts. The sap was squeezed from the trunk and used directly for lipid production. High lipid accumulating oleaginous yeasts were screened using sap.

Materials and methods

Preparation of sap and hydrolysis of fiber from oil palm trunk

Oil palm trunk, approximately 25 years old, was obtained from the Pure Parawood Co., Ltd. (Suratthanee Province, Thailand). The old palm trunk was cut up into pieces and then ground by a blending machine. The sap was squeezed by compressing the disks using a laboratory-scale press. The sap was filtered with cheesecloth and concentrated using evaporation to obtain the desired sugar concentration.The pH was adjusted to 6.0. The sap, with a total sugar concentration of 20 g/L was used directly for lipid production.

Microorganisms and media

Six oleaginous yeasts including *Trichosporonoides spathulata* JU4-57,*Kluyveromyces marxianus* X32, *Candida tropicalis* X37, *Rhodotorula mucilaginosa* G43, *Yarrowia lipolytica* TISTR 5151 and *Yarrowia lipolytica* TISTR 5054 were obtained from the Bioprocess Engineering Laboratory (Faculty of Agro-Industry, Prince of Songkla University).Plate cultures were incubated at room temperature (30±2

°C)for 24 h. The cells were then transferred to 125 mL Erlenmeyer flasks containing 50 mL of culture medium comprised of glucose 4 %, peptone 0.5 %, yeast extract 1.5 %, pH 6.0. The flasks were incubated at room temperature (30±2 °C) on a rotary incubator shaking at 140 rpm for 24 h for the seed culture.

Culture conditions

Selection of oleaginous yeasts

The cultures were initiated with 10 % of a 24 h old seed culture (approximately 10^7cells/mL) into the medium adding with and without ammonium sulfate (0.5 g/L). The cultures were incubated on a rotary shaker at 140 rpm at room temperature (30±2 °C) for 72 h. Each set of experiments were sampled every 12 h. The samples were centrifuged at a speed of 7,000 rpm for 10 minutes to separate supernatant and the dried cell weight and lipids were determined. The growth and lipid production of each yeast were compared.

Optimization of medium components and culture conditions

The infect of addition of ammonium sulfate (0.5 g/L) (w/w) and sugar concentration (20, 40 and 60 g/L) on biomass and lipid production of the selected yeasts were investigated. The precultured inoculums were transferred into 50 mL sterilized medium in a 250-mL Erlenmeyer flask and incubated at room temperature for 3 days on rotary shaker at 140 rpm.

Fed-batch fermentation

In the fed-batch fermentation, the start-up of the process was the same as that for batch operation. The feeding started in the middle of the exponential phase in which 20 g/L sugar was added at 0 h, 36 h, 48 h and 60 h. The time courses of biomass, lipid yield production were determined.

Analytical methods

Biomass concentration was determined gravimetrically. Samples containing 2 mL fermentation broth withdrawn from the flasks were centrifuged at 7,000 rpm for 10 min. The cell pellets were collected and washed twice with distilled water and then dried at 60°C to constant weight (Papanikolaou *et al*., 2002). The dry biomass was ground into a fine powder. The powder was blended with 1 mL chloroform: methanol (2: 1) and the mixture was sonicated for 30 min at 70 kHz and room temperature. Solvent phase was recovered by centrifugation. The process was repeated two more times. The extracted lipid were recovered by evaporating the solvent. Lipid content was expressed as gram lipid per gram dry biomass (Xue *et al*., 2008). All experiments were performed in triplicates. Analysis of variance was performed to calculate significant differences in treatment means, and the least significant difference ($p \leq 0.05$) was used to separate means, using the SPSS software.

Results and Discussion

Selection of oleaginous yeasts

Six oleaginous yeasts *T. spathulata* JU4-57, *K. marxianus*X32, *C. tropicalis* X37, *R. mucilaginosa* G43, *Y. lipolytica* TISTR 5151 and *Y. lipolytica* TISTR 5054 were cultivated in the sap broth with and without addition of ammonium sulfate (0.5 g/L) as additional nitrogen source. The results are shown in Fig. 1 and Fig. 2. *C. tropicalis* X37 and *R. mucilaginosa* G43 could growth better with the addition of additional nitrogen source. The obtained

biomass were 11.05 and 9.75 g/L,respectively. The maximum lipid yield and lipid content of *C. tropicalis* X37 were 0.96 g/L and 14.12% and those of *R. mucilaginosa* G43 were 9.75 g/L and 1.0 g/L,

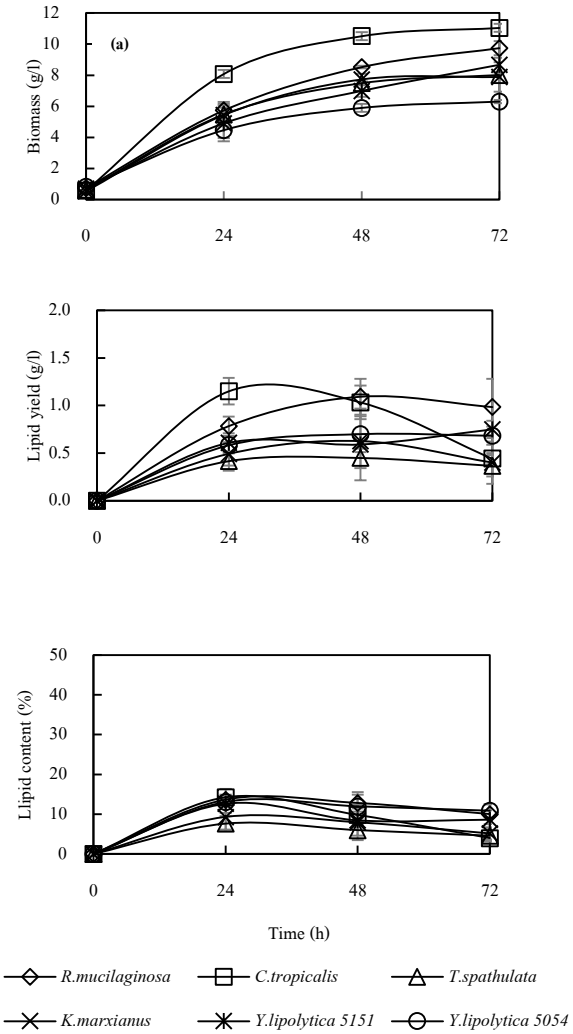

Figure1 Biomass, lipid production and lipid content by oleaginous yeasts cultivated in sap broth withaddition ofnitrogen source. (a) biomass, (b) lipid yield, (c) lipid content.

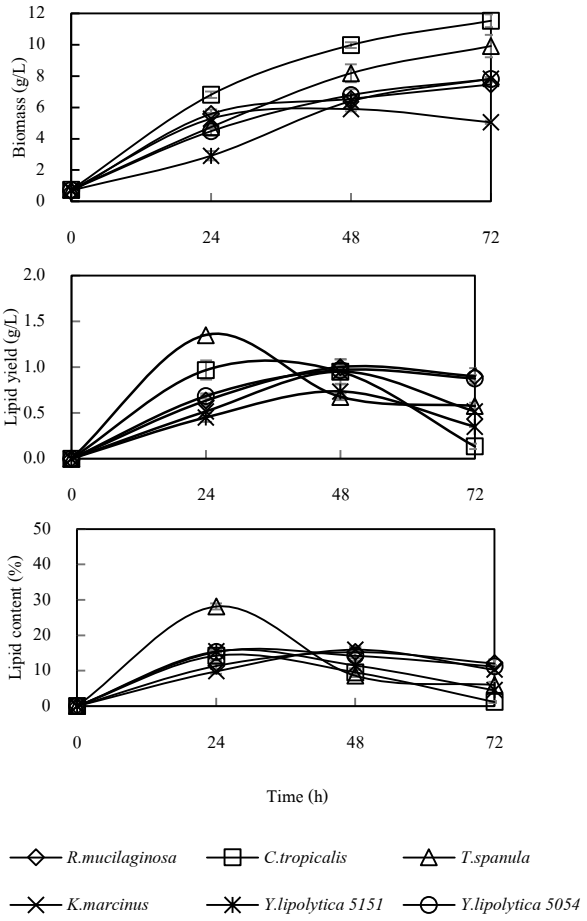

Figure2 Biomass, lipid production and lipid content by oleaginous yeasts cultivated in sap broth without addition ofnitrogen source. (a) biomass, (b) lipid yield, (c) lipid content.

Effect of sugar concentration

To improve for lipid production by *C. tropicalis* X37 and *R. mucilaginosa* G43, the suitable sugar concentration was determined. The concentration of sugar was varied at 20, 40 and 60 g/L. As shown in Fig. 3, With increasing sugar concentration from 20 g/L to 40 g/L, the biomass slightly increase from 8.83 t0 8.50 g/L and lipid production increase from 0.75 to 1.35 g/L with the increase in lipid content may reduce from 25.01 to 25.34%. When increase sugar concentration up to 60

g/L, the biomass further increase up to 9.12 g/L but the lipid yield decrease to 1.15 g/L. Fig.4 shows Effect of sugar concentration on cell growth and lipid accumulation of *R.mucilaginosa* G43. With increase sugar concentration from 20 g/L into 40 and 60 g/L, the biomass increase from 7.10 up to 8.50 and 8.62 g/L. but the sugar concentration at 40 g/L gave the highest lipid yield of 1.40 g/L. The nitrogen concentration also increase and reduce the accumulation of lipid. The high sugar concentration could also introduce a considerable glucose inhibitory effect (Leesing *et al*., 2011). It has been reported that there is the suitable nitrogen source for lipid production with increase sugar concentration in the sap.

When to an influx of excess carbon from the medium, which has a high C/N ratio, resulting in a decrease in biomass production and high levels of lipid production, but results from a further increase in C/N ratio, leading to the repression of lipid accumulation in favor of secondary metabolite production. If the desired end product of the process is lipid, then the process must be designed so as to ensure the maximal conversion of the carbon taken up into lipids, by minimizing by-product (citric acid) production and maximizing lipid synthesis (Papanikolaou *et al*., 2002).

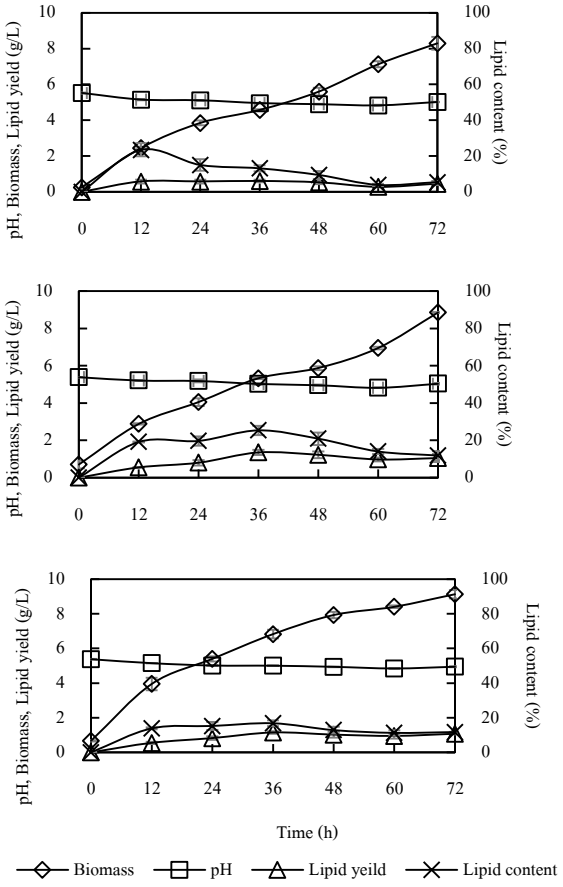

Figure 3 shows Effect of sugar concentration on cell growth and lipid accumulation of *C. tropicalis* X37(a) 20g/L, (b) 40 g/L, (c) 60 g/L.

Effect of fed-batch fermentation

Fed-batch fermentation modes have been widely applied for microbial lipid production. The fed-batch culture method was performed to reduce the inhibitory effects of a high sugar concentration. The additional glucose was added at 36, 48 and 60 h to keep C/N ratio at a high level to enhance the lipid production. Fig. 5 presents the typical time-course of sugar concentration, biomass and of *C. tropicalis* X37 and *R. mucilaginosa* G43. The overall lipid yield and lipid content were increased up to 2.85 g/L and 40.07% respectively. The lipid yield and lipid content of *R. mucilaginosa* G43 were increased up to 2.10

g/L and 53.02% , respectively. It could then be concluded that the fed-batch fermentation could enhance lipid production by keeping carbon source at a constantly high level, namely high C/N ratio.

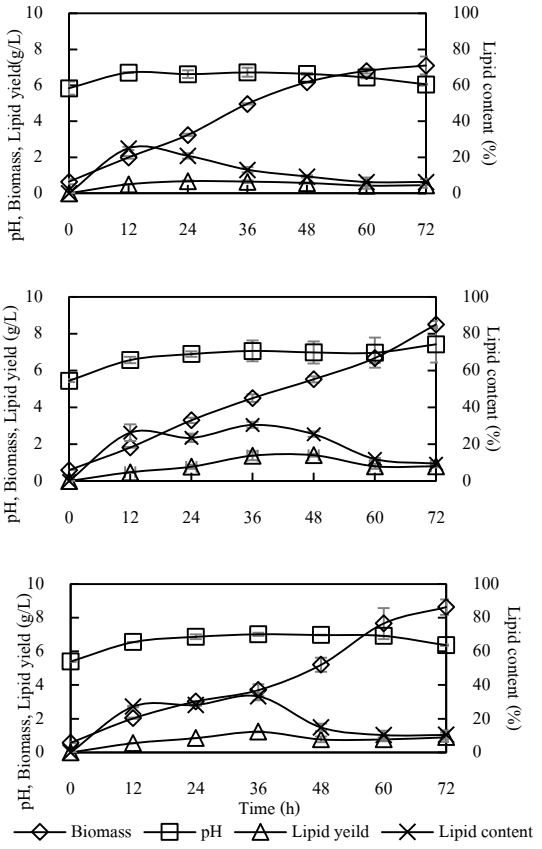

Figure 4 shows Effect of sugar concentration on cell growth and lipid accumulation of*R.mucilaginosa*G43(a) 20g/L, (b) 40 g/L, (c) 60 g/L.

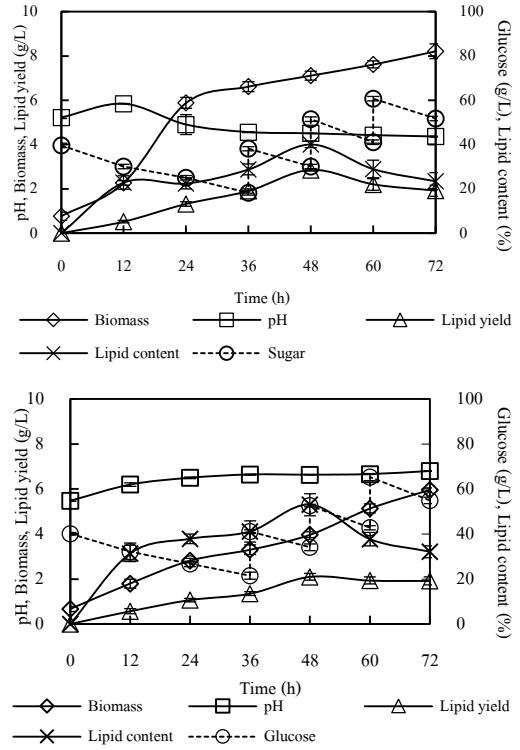

Figure 5 Fed-batch fermentation of *C. tropicalis* X37 (a) and *R.mucilaginosa* G43 (b). 20 g/L glucose was added at 36 48 and 60 h

Conclusion

This study has shown that oil palm sap can be used as an inexpensive renewable substrate for lipid production. Among six strains tested, *C. tropicalis* X37 and *R.mucilaginosa* G43 gave the highest lipid production in the sap without addition of ammonium sulfate. The optimal initial sugar concentration in the sap for lipid production was 40 g/L.The fed-batch fermentation with the addition of glucose gave higher lipid production due to the higher C/N ratio than the batch fermentation.

Acknowledgement

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