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Screening of Oleaginous Yeast for Lipid Production Using Rice Residue from Food Waste as a Carbon Source

Wimada Srisuwan[1], Charin Techapun[2], Phisit Seesuriyachan[2], Masanori Watanabe[3] and Thanongsak Chaiyaso[2,]*

[1]*Biotechnology Program, Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand*
[2]*Bioprocess Research Cluster (BRC), Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand*
[3]*Faculty of Agriculture, Yamagata University, Yamagata, Japan*
[*]*Corresponding author: thachaiyaso@hotmail.com*

Abstract

Rice residue from food waste contained of starch as a mainly component which could be either hydrolyzed to be fermentable sugars or directly used as a carbon source for the growth and high value metabolites production by various microorganisms. Therefore, this study focused on the utilization of rice residue and rice residue hydrolysate from food waste as a carbon source for the growth and lipids production of oleaginous yeast. Rice residue obtained from canteen of the Faculty of Agro-Industry, Chiang Mai University, Thailand. It composed of moisture content (76.68±0.55%), crude fat (1.76±0.47%), crude protein (3.04±0.06%), ash content (0.46±0.07%), and carbohydrate content (18.05±0.01%), respectively. Rice residue was then subjected to enzymatic hydrolysis using α-amylase and amyloglucosidase (AMG), resulting the maximal reducing sugars of 168.02±0.02 g/L. The screening of oleaginous yeast from flowers and leaves samples from Doi-Inthanon National Park, Faculty of Agro-Industry, Chiang Mai University, the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR) and the Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University were investigated. Sixty-seven isolates were obtained, and only four isolates were identified as oleaginous yeast because of containing high lipids content more than 20% (w/w), when glucose or rice residue hydrolysate was used as a carbon source. Those oleaginous yeasts were identified as *Rhodotorula* sp. C7, *Rhodosporidium paludigenum* C10, and the new isolate TC32, respectively. Their growths and lipid productions were compared with *Diozegia* sp. TISTR5792. The results showed that, C7, C10, TISTR5792 and TC32 produced the maximal lipids content of 24.26±0.56, 23.69±0.91, 22.43±1.09 and 23.07±0.80% (w/w) when cultivated in the basal medium supplemented with enzymatic-rice residue hydrolysate. Surprisingly, we found that TISTR5792 and TC32 could grow well in the medium supplemented with rice residue

(without hydrolysis) and showed lipids content of 18.41±0.10 and 21.67±0.02% (w/w), respectively. These results indicated that rice residue from food waste shows a high potential to be an effective carbon source for the growth and lipid production of the selected oleaginous yeasts.
Keywords : *rice residue hydrolysate, food waste, oleaginous yeast, lipids*

1. Introduction

Food waste, an organic solid waste which is usually discharged from various sources including canteen, restaurants, commercial kitchens and cafeterias (1). The amount of food waste has been predicted to increase in the next 25 years due to economic and population growth, mainly in Asian countries. For example, the annual amount of food waste in Asian countries could increase from 278 to 416 million tons from year 2005 to 2025 (2). There are usually landfilled or incinerated which can produce many environmental problems (3) such as emission of greenhouse gases especially methane and carbon dioxide (4). The major components of the food waste is starch, which can be hydrolyzed to fermentable sugars (5) The fermentable sugars can be used as a carbon source for the growth and high value metabolites productions e.g. ethanol (3, 5-8), hydrogen gas (H_2) (9-11), methane (12, 13), and microbial oil a feedstock for biodiesel by various oleaginous microorganisms.

Oleaginous yeasts are a single cell oil (14) which can be fast synthesized and accumulated lipid in their cell more than 20% (w/w). Lipids derived from oleaginous yeast, known as microbial lipid, have fatty acid compositions similar to vegetable oil which can use as a feedstock for biodiesel production. The production of microbial lipid has many advantages more than vegetable oils such as shorter culture period, easy to harvest and no need of agricultural land (15). In addition, oleaginous yeast also can utilize various of low cost substrates (16) such as crude glycerol (15, 17), molasses (16, 18), hydrolysate from wheat straw (19), soluble starch (20), cassava starch hydrolysate (21) and palm oil mill effluent or POME (22).

The objective of this study is to screen oleaginous yeasts which are capable to use rice residue and rice residue hydrolysate from food waste as a carbon source for the growth and lipids production. Moreover, this research demonstrated the high efficiency method using the bioconversion of food waste to be added-value microbial oil which can be used as a substrate for biodiesel production.

2. Materials and methods

2.1 Raw material

Rice residue was obtained from canteen of the Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand, during the first semester of the academic year 2015. It was crushed into small size by using a blender. After that, it was frozen at -20ºC until used. Proximate analysis of rice residue was analyzed according to the AOAC 2002 (23). The composition of rice residue e.g. moisture content, crude fat, ash content and crude protein is provided in Table 1.

Table 1. Composition of rice residue from food waste

Parameters	Composition (%g/g)
Moisture content	*76.68±0.55
Crude fat	1.76±0.47
Ash	0.46±0.07
Crude protein	3.04±0.06
Carbohydrate (by difference)	18.05±0.01

*Means and standard deviations of triplicate samples

2.2 Enzymatic hydrolysis of rice residue from food waste

Rice residue from food waste was used as a substrate for fermentable sugars production. The substrate was subjected to enzymatic hydrolysis by mashing with distilled water and pH was adjusted to be 4.5 by adding 10% H_2SO_4. Then, 183.17 U/g rice residues or 10% (v/w) of α-amylase (SPEZYME FRED; Genencor, USA) was added, and the reaction was carried out at 80ºC. After 2 h of reaction time, 116.67 U/g rice residues or 1.5% (v/w) of AMG (DISTILLASE VPH; Genencor, USA) was added and incubated at 60ºC for 72 h. The releasing of reducing sugar was measured using dinitrosalicylic acid (DNS) method (24).

2.3 Acid hydrolysis of residue from food waste

For the acid hydrolysis, the rice residue was mixed with 3.0 M HCl at the solid to liquid ratio of 7:3 before autoclaving at 121ºC for 15 min. After that, the pH of hydrolysate was adjusted to neutral pH (6.5-7.5) by adding 2.5 M NaOH. The reducing sugar content of hydrolysate was measured using DNS method (24).

2.4 Screening and isolation of oleaginous yeast for lipid production

Oleaginous yeasts were screened from flowers, fruits and leave samples obtained from Doi-Inthanon National Park, Faculty of Agro-Industry, Chiang Mai University and Thailand Institute of Scientific and Technological Research (TISTR), Thailand. They was enriched in yeast-malt extract medium (YM) containing (per liter); yeast extract 4.0 g, malt extract 10.0 g and glucose 4.0 g supplemented with 100 ppm chloramphenicol to minimize bacterial growth. The initial pH was adjusted to 6.0 with H_3PO_4 or 0.1 M KOH and then, autoclaved at 121ºC for 15 min. All of samples were incubated on incubator shaker (Kühner, Switzerland) at 28ºC, with shaking speed 200 rpm for 3 days. After that, the culture broth was diluted by 10-folds serial dilution technique and spread on YM medium agar plate. The yeasts colonies were selected and re-streaked on YM medium agar plate. The lipids accumulated in yeast cell was selected by Sudan black B technique (25). Moreover, the pure yeasts isolates was kept on YM slant at 4ºC or maintained in 60% glycerol stock at -20ºC until used (26).

2.5 Screening of oleaginous yeast using rice residue from food waste as a carbon source

The glycerol stock of yeast isolate (from selection 2.4) was transferred into 250 mL Erlenmeyer flasks containing 50 mL of YM on incubator shaker at 28ºC with a shaking speed of 200 rpm for 3 days. The starter culture was 10.0% (v/v) inoculated by batch fermentation. They were cultivated in basal medium supplemented with either glucose, enzymatic or acid-hydrolysate from rice residue, soluble starch (Sisco Research Laboratories Pvt. Ltd., India) or rice residue. The concentrations of carbon source in each experiment were adjusted to be 10.0 g/L as glucose content in each carbon source. The glucose content in each carbon source was measured by phenol-sulfuric method (27). The basal medium contained (per liter) of yeast extract 1.0 g, KH_2PO_4 5.5 g, $(NH_4)_2SO_4$ 5.3 g, K_2HPO_4 3.7 g, $MgSO_4.7H_2O$ 0.5 g, $MnSO_4.H_2O$ 0.2 g and NaCl 0.5 g (26). The initial pH was adjusted to 6.0. The culture was incubated on an incubator shaker at 28ºC with a shaking speed of 200 rpm for 5 days.

2.6 Analytical method

Dry cell weight (DCW) was collected from 5 day-olds cultivation broth, which was taken from each flask and centrifuged at 6,000 rpm for 10 min. The cell pellet was washed twice with distilled water before drying at 80ºC overnight and transferred to desiccator until constant weight (26).

The lipids of cell pellet was extracted by a modified method of Bligh and Dyer (25), which broke the yeast cell, carried out in screw cap tube (25×150 mm) with a mixture of chloroform : methanol (2:1, v/v) and glass beads (size 3 mm). The mixture was vigorously shaken in a vortex mixer for 30 min, and then sonicated for 15 min. The ruptured cell and extracted lipids were centrifuged, and the clear supernatant was collected and removed by vacuum evaporator. After that, crude lipid was transferred to desiccator until constant weight (g/L). The lipids content was expressed in the percentage of the crude lipid in relation to the dry cell weight (% g/g).

3. Results and discussion

3.1 The enzymatic and acid hydrolysis of rice residue from food waste

The enzymatic-rice residue hydrolysate contained reducing sugars of 168.02±0.02 g/L and the product yield coefficient (p/s) of 0.960 g/g, while acid hydrolysis method showed 128.55±0.04 g/L and the product yield coefficient of 0.734 g/g, respectively. These results indicated that enzymatic hydrolysis yielding high content of glucose because of the specificity of amylolytic enzymes (28). The acid hydrolysis showed lower reducing sugar than enzymatic method. The main drawback of acid hydrolysis is formation of undesired products e.g. furans, carboxylic acid and phenolic compound under high temperature and pressure conditions (19). Moreover, those byproducts have been reported as the microbial growth inhibitors (29).

3.2 Screening and isolation of oleaginous yeast for lipid production

The screening of oleaginous yeast from flowers and leaves samples and the culture collections of TISTR and the Division of Biotechnology, Faculty of Agro-Industry were studied. Sixty-seven of yeast isolates were obtained. After cultivation in the basal medium supplemented with glucose as a carbon

source, found that seventeen isolates could accumulate lipids in their cell more than 10% (w/w) as shown in Figure 1. However, only 2 strains of *Rhodotorula* sp. C7 and *Rhodosporidium paludigenum* C10 could accumulate lipid in theirs cell more than 20% (w/w) and produce maximum lipids content of 22.44±1.08 and 21.58±0.05% (w/w), respectively.

3.3 Screening of oleaginous yeast using rice residue hydrolysate from food waste as a carbon source

Seventeen isolates (selected from section 3.2) were cultivated in basal medium supplemented either enzymatic or acid-rice residue hydrolysate.

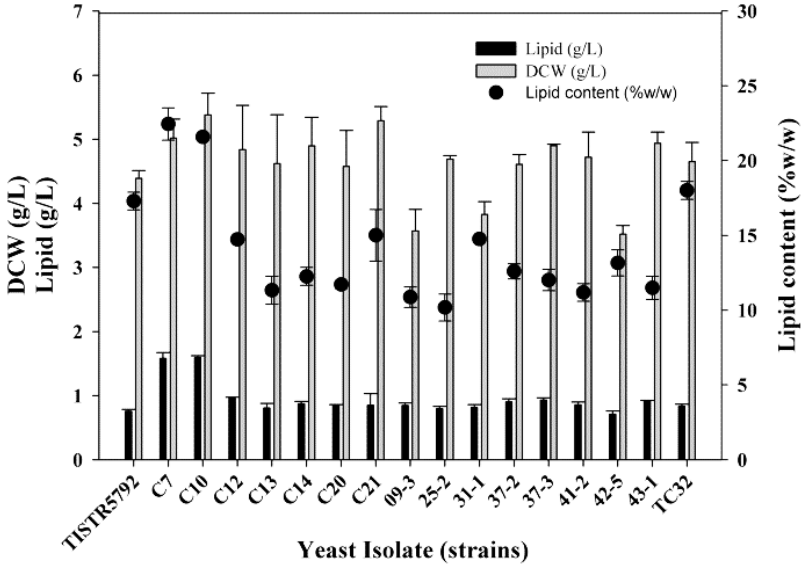


Figure 1. Screening of oleaginous yeasts using glucose as a carbon source

The results from Figure 2 showed that, *Rhodotorula* sp. C7, *Rhodosporidium paludigenum* C10, *Diozegia* sp. TISTR5792 and the newly isolate TC32 could produce the maximal lipids content of 24.26±0.56, 23.69±0.91, 22.43±1.09 and 23.07±0.80% (w/w), respectively, when cultivated in the basal medium supplemented with enzymatic-rice residue hydrolysate. While, cultivation in basal medium supplemented with acid-rice residue hydrolysate showed the lipids content of 21.84±0.56, 22.41±0.23, 19.32±0.80 and 19.00±0.50% (w/w) by *Rhodotorula* sp. C7, *Rhodosporidium paludigenum* C10, *Diozegia* sp. TISTR5792 and the newly isolate TC32, respectively (Figure 3).

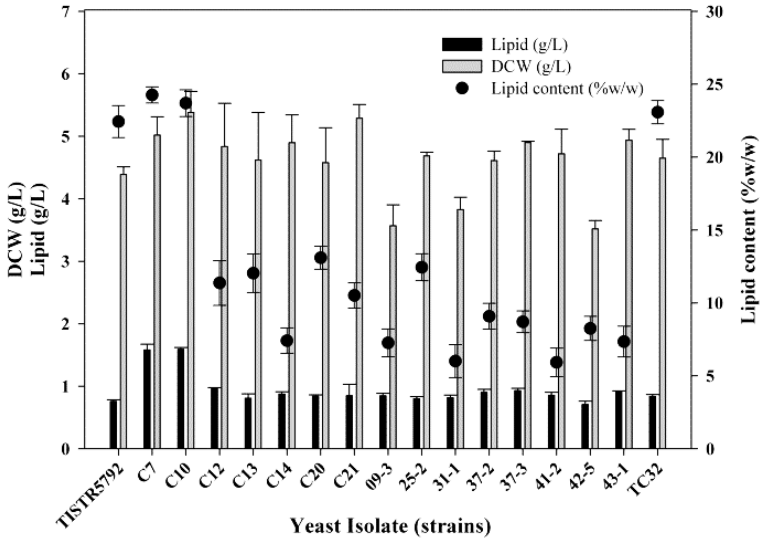


Figure 2. Screening of oleaginous yeasts using enzymatic-rice residue hydrolysate as a carbon source

From these results we found that enzymatic-rice residue hydrolysate showed lipids production higher than glucose and acid hydrolysis. It might be that rice residue from food waste contained not only carbohydrate, but other component also found in this starchy material (Table 1). Crude lipid, crude fat and some trace elements in term of ash content may enhance the growth and lipid production of oleaginous yeast. Similar with the report of Subramaniam et al. (30), who found that accumulation of lipids in yeast cell takes place under conditions of limitations caused by a nutrient other than carbon source.

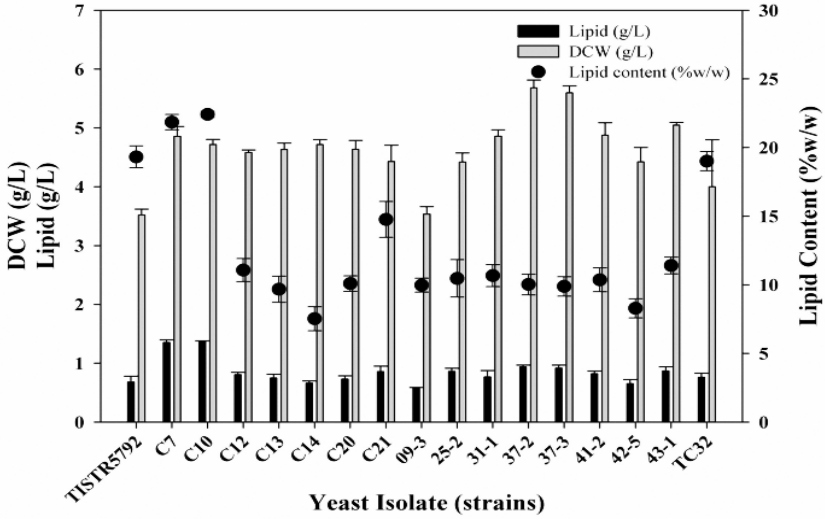


Figure 3. Screening of oleaginous yeasts using acid-rice residue hydrolysate as a carbon source

Moreover, cultivation in basal medium supplemented with enzymatic-rice residue hydrolysate showed higher lipid content than acid-rice hydrolysate. It might be that acid-rice hydrolysate may contain furfural or HMF which usually occurred during heat-process and acid hydrolysis. These compounds have been reported as the inhibitor of microbial growth by reducing enzymatic and biological activities, leading to low productivity (31).

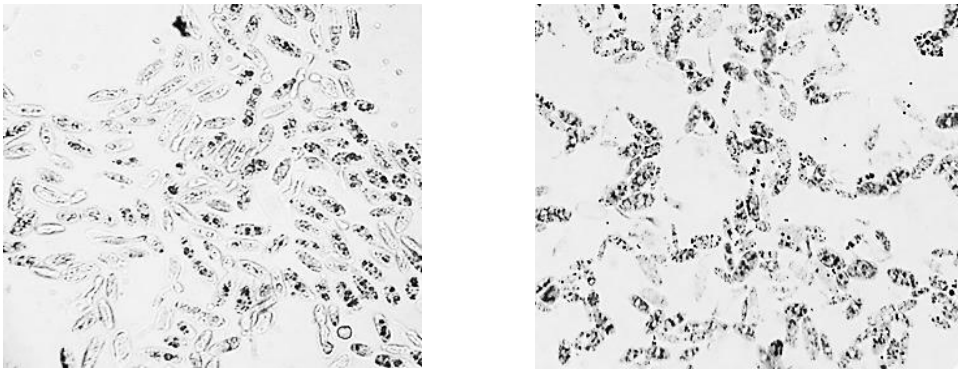
3.4 Screening of oleaginous yeast using rice residue from food waste as a carbon source

The ability of directly bioconversion of rice residue to biomass and lipids of seventeen isolates (selected from section 3.2) were also investigated. The results revealed that only two isolates of *Diozegia* sp. TISTR5792 and the newly isolate TC32 could use rice residue for theirs growth and accumulated the maximum lipid content of 18.00±0.83 and 21.67±0.02% (w/w), respectively. Similar with the result of Wild et al. (32), who reported that the lipid production yields from *Lipomyces starkeyi* on starch was higher than glucose. The ability of directly convert of starch and rice residue was confirmed by the α-amylase and AMG activities as presented in Table 2. The results found that *Diozegia* sp. TISTR 5792 and newly isolate TC32 could produce extracellular amylolytic enzymes with α-amylase activities of 0.25±0.18 and 0.54±0.09 U/mL and AMG activities of 0.020±0.000 and 0.023±0.000 U/mL, respectively. Moreover, the lipid accumulation of these two strains were further confirmed by staining with Sudan black B technique (25). The high intensity of black color indicating high content of lipid which accumulated in yeast cell (Figure 4) (33).

Table 2. Characteristics of oleaginous yeast TC32 and TISTR5792 when cultivation in basal medium supplemented with rice residue from food waste as a carbon source

Characteristic	Isolate TC32		TISTR5792	
	Soluble starch	Rice residue	Soluble starch	Rice residue
DCW (g/L)	2.49±0.22*	5.82±0.30	3.37±0.38	5.29±0.30
Lipid (g/L)	0.63±0.07	1.26±0.01	0.70±0.21	0.97±0.01
Lipid content (%w/w)	24.00±1.70	21.67±0.02	21.65±3.90	18.41±0.83
α-Amylase activity (U/mL)	0.14±0.04	0.54±0.09	0.21±0.10	0.25±0.18
AMG activity (U/mL)	0.036±0.00	0.023±0.00	0.031±0.00	0.020±0.00

*Means and standard deviations of triplicate samples



(A) (B)

Figure 4. Staining of lipid in cell of oleaginous yeast by Sudan black B technique (A) TISTR5792 and (B) newly isolate TC32, the following images were taken at 400x

Conclusion

Rice residue from food waste could be used as a carbon source for the growth and lipid production via a bioconversion by some oleaginous yeast. The conventional method needs 2 steps of hydrolysis of starchy material to be fermentable sugars and fermentation. The disadvantage of this method is requirement of expensive commercial amylolytic enzymes. So, the result obtained in this study indicating that the amylolytic producing oleaginous yeast, TC32 which isolated from flower samples obtained from Doi-Inthanon National Park, Chiang Mai, Thailand, could overcome the disadvantage of the traditional method by no need an expensive enzymes and showing high ability to directly convert starchy material to biomass and lipid.

Acknowledgments

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