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Use of low-cost substrates for cost-effective production of extracellular and cell-bound lipases by a newly isolated yeast *Dipodascus capitatus* A4C

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ABSTRACT

As the enzyme production and its application is a costly process mainly because of the inducible production, this study aimed to screen the basic components of media for the production of cost-effective lipases. A newly isolated yeast strain, namely *Dipodascus capitatus* A4C is a specific yeast that could produce lipases in both extracellular and cell-bound forms. Various low-cost substrates were applied as single or mixed carbon sources for *D. capitatus* A4C. These included non-edible vegetable oil (*Jatropha curcas* oil), crude glycerol, and molasses. The suitable cost-effective carbon source for extracellular lipase production was 2% *J. curcas* oil, which gave high extracellular lipase activity of 2670 U/L at 96 h. While the suitable low-cost carbon source for cell-bound lipase production was 2% molasses, which gave high cell-bound lipase activity of 373 U/L at 48 h. The present work emphasized that the lipolytic activity of *D. capitatus* A4C can be improved with a compatible use of low-cost carbon sources as co-substrates.

1. Introduction

Lipase (triacylglycerol acylhydrolase, EC: 3.1.1.3) is an omnipresent enzyme which can hydrolyze and synthesize ester bonds (Lukovi et al., 2011). There are different sources of lipases such as plant, animal, and microbial lipases. As compared to plant and animal lipases, though animal and plant lipases were used for industrial production before this era microbial lipase has gained more attraction in the industrial as well as scientific community (Treichel et al., 2010). This is because the microorganisms have high ability to produce lipases, they are single cell entity, easily grown and manipulated conventionally and genetically (Shimada et al., 1999).

Even though the lipase production can partially be induced with the help of fatty acids, yet the lipase production is basically a constitutive gene expression (Ban et al., 2001; Fabiszewska et al., 2014; Yan et al., 2014a,b). The presence of these constitutive lipases assists microorganisms in degrading oils and fatty acids thus consequently mass production of lipases. The reported lipase production conducted in *Aspergillus*, *Calvatia*, *Rhizopus*, and *Rhodotorula* revealed that lipase production is a constitutive production, independent of lipidic substrates added to the fermentative medium, even though their presence may increase the lipolytic activity. Whereas the research on lipase production by *Thraustochytrium* spp. indicated that lipidic substrates are essential for lipase production (Usha and Muraleedharan, 2011). Therefore, it is important to design the specific and suitable medium components for constitutive and inducible lipase production by specific microorganisms.

Different culture parameters such as carbon sources (Fabiszewska et al., 2015), nitrogen sources (Almeida and Taulk-tornisielo, 2013), inducers (Coelho, 2017), initial pH, temperature (Ire and Ike, 2014) and aeration (Potumarthi and Subhakar, 2008) are basically studied to enhance the microbial lipase production. Various expensive acidic substrates and fatty acids are used as inducers for the microbial lipase, which hike up the prices of the microbial lipases. Edible oils are mostly used as either the main carbon sources or as the inducers but in both cases, in one hand, the production cost of the lipase increases and on

Abbreviations: ECL, Extracellular lipase; CBL, Cell-bound lipase; CBM, Cell biomass; LPM, Lipase producing medium; LPM-G, Lipase producing medium with glucose; LPM-G-JCO, Lipase producing medium with glucose and *Jatropha curcas* oil; LPM-G-PO, Lipase producing medium with glucose and palm oil; LPM-JCO, Lipase producing medium with *J. curcas* oil; LPM-PO, Lipase producing medium with palm oil; LPM-M, Lipase producing medium with molasses; LPM-M-JCO, Lipase producing medium with molasses and *J. curcas* oil; LPM-CG, Lipase producing medium with crude glycerol; LPM-CG-JCO, Lipase producing medium with crude glycerol and *J. curcas* oil

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the other hand, the competition between the food industries and enzyme industries occurs and this effects the prices of human diet and food. Low-cost carbon sources and non-edible oils may help in cost reduction and further development of the microbial lipases (Caroline et al., 2014; Coelho, 2017; Potumarthi and Subhakar, 2008). Nitrogen sources are essential for the growth and lipase production. Montesinos et al. (1996) reported that the lipase production by *Candida rugosa* in batch and continuous fermentation process was higher when ammonium nitrate was used as a nitrogen source rather than urea.

In the present study, we aimed to screen a set of locally available low-cost substrates as promising cost-effective media for lipase production by a newly isolated yeast identified as *Dipodascus capitatus* A4C. This lipase-producing yeast strain was isolated as the most potent lipase producer from sample sources of palm oil contaminated wastes, which from the previous study were collected from two palm oil mills in Satun, a small southern Thai province. Organic and inorganic nitrogen sources as well as cost-effective carbon sources were screened for the most cost-efficient and suitable medium for lipase production. Molasses from sugar industry, crude glycerol from biodiesel production plant, and non-edible *Jatropha curcas* oil were selected as low-cost carbon sources. Yeast extract, peptone, and urea were selected as the organic nitrogen sources, whereas ammonium nitrate was applied as an inorganic nitrogen source. The effects of these mixed carbon and nitrogen sources on cell growth, extracellular and cell-bound lipases production, in both inducible and constitutive forms were investigated.

2. Materials and methods

2.1. Materials

The commercially-available refined palm oil (Morakot) was obtained from local supermarket. The non-edible *Jatropha curcas* oil was kindly provided by the Center of Excellence for Jatropha, Kasetsart University, Bangkok, Thailand. Molasses was purchased from a retailer located in Songkhla province, Thailand. Crude glycerol was a gift from the biodiesel production plant, Faculty of Engineering, Prince of Songkla University. All other chemicals used were of analytical grade and were supplied by Loba Chemie (India), Thermo Fisher Scientific, Inc. (USA), Ajax Finechem (Australia), Nacalai Tesque, Inc. (Japan), and RCI Labscan Co., Ltd (Thailand). Various components of culture media were supplied from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

2.2. Microbial strain and culture media

Yeast strain *Dipodascus capitatus* A4C (GenBank accession number MF135608) was obtained from the Molecular Biotechnology Laboratory, Department of Industrial Biotechnology, Faculty of Agro-industry, Prince of Songkla University, Thailand. Yeast malt medium (YM) containing 10% (w/v) glucose, 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, and 0.3% (w/v) malt extract was used as broth medium. For YM agar medium, the YM was added with 1.5% (w/v) bacteriological agar. Lipase production medium (LPM) containing either, 2% (w/v) palm oil (LPM-PO), 2% (w/v) *J. curcas* oil (LPM-JCO), 2% (w/v) glucose (LPM-G), 2% (w/v) molasses (LPM-M), and 2% (w/v) crude glycerol (LPM-CG) as main carbon source or combined carbon sources including 1% (w/v) glucose and 1% (w/v) palm oil (LPM-G-PO), 1% (w/v) glucose and 1% (w/v) *J. curcas* oil (LPM-G-JCO), 1% (w/v) molasses and 1% (w/v) *J. curcas* oil (LPM-M-JCO), and 1% (w/v) crude glycerol and 1% (w/v) *J. curcas* oil (LPM-CG-JCO) with the respective carbon source at a final concentration of 2% (w/v). The other components of LPM were: 0.4% (w/v) NH_4NO_3, 0.47% (w/v) KH_2PO_4, 0.03% (w/v) $Na_2HPO_4.12H_2O$, 0.1% (w/v) $.MgSO_4.7H_2O$, 0.001% (w/v) $.FeSO_4.7H_2O$, 0.001% (w/v) $.CaCl_2.2H_2O$, 0.001% (w/v) $.MnSO_4.4H_2O$, 0.01% (w/v) yeast extract, and 0.2% (w/v) gum arabic; pH 7.0. To study the effect of nitrogen sources in LPM-G, 0.4% (w/v) NH_4NO_3 was replaced with 0.4% (w/v) urea, 0.4% (w/v) peptone, and 0.4% (w/v) yeast extract, respectively.

2.3. Methods

2.3.1. Lipase production on various substrates

For seed culture preparation, one loop full of each single colony of *D. capitatus* A4C growing on YM agar plate after incubation at room temperature (30 ± 2 °C) for 72 h was transferred into 250 mL *Erlenmeyer* flask containing 10 mL of YM broth. After 24 h of incubation on the rotary shaker (200 rpm) at 30 ± 2 °C, the total 10 mL of seed culture was inoculated into 250 mL *Erlenmeyer* flask containing 90 mL of YM broth. After 72 h of incubation on the same condition, 10% of the seed culture was inoculated into 250 mL *Erlenmeyer* flask containing 90 mL of lipase production medium. The culture was then incubated at 200 rpm for 120 h at 30 ± 2 °C. Finally, the culture broth was centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant was used to measure the extracellular hydrolytic activity of the lipase. While the cell pellets were washed twice with 0.1 M phosphate buffer (pH 7.0) and were used in measuring the cell-bound lipase activity.

2.3.2. Lipolytic activity assay

Both extracellular and cell-bound lipases (ECL; CBL) hydrolytic activity were determined by using the cupric acetate method following the procedure of Lee and Rhee (1993) with slight alterations. 5% (w/v) cupric acetate solution was prepared and pH was adjusted to 6.1 by the addition of pyridine. To perform the reaction in a two-phase system, the reaction was prepared by taking 0.5 mL of phosphate buffer (0.1 M; pH 7.0) mixing it with 1 mL of 10% (v/v) palm oil in isooctane, and 0.2 mL of culture supernatant for extracellular lipase activity measurement. While for cell-bound lipase hydrolytic activity assay, cell pellets from 1 mL of culture broth was harvested and treated as stated above for the extracellular lipase activity measurement. The reaction was incubated on rotary shaker at 300 rpm for 30 min at 30 ± 2 °C. The enzyme reaction was stopped by adding 0.3 mL of 6 N HCl. After thoroughly mixing, 1 mL layer of upper isooctane was drawn and mixed with 0.4 mL of cupric acetate solution. The dissolved free fatty acid in isooctane layer was then determined by measuring the absorbance at 715 nm against the control using UV-1800 spectrophotometer (Shimadzu Corp., Japan). The standard curve of palmitic acid was also prepared. The hydrolytic activity of the enzyme was determined by measuring the amount of released free fatty acids using the standard curve of palmitic acid as a reference scale. One unit of enzyme activity is defined as the enzyme vital for the release of 1 μmol of palmitic acid per unit time under specified conditions.

2.3.3. Biomass determination

To determine biomass concentration, the cell pellets were prepared by centrifugation 10 mL of the culture broth at 10,000 × *g* for 15 min at 4 °C. The cell pellets were then washed twice with 0.85% (w/v) NaCl solution and were dried at 70 °C in the universal oven UF110 (Memmert GmbH + Co. KG) until the constant weight was obtained. Total dry cell mass (g/L) was calculated according to the formula below:

Total dry cell mass (g/L) = Dry cell weight (g/10 mL) × 100

2.3.4. Total carbohydrate determination

The total carbohydrate content in molasses was estimated according to the phenol-sulfuric acid method as described by Dubois et al. (1956). The total 55% of glucose (0.55 g/g of molasses) was determined in the molasses used in this study. 1.82 and 3.64 g of molasses were added to LPM to obtain the final 1% and 2% (w/v) of glucose, respectively.

2.3.5. Total glycerol determination

The total glycerol in crude glycerol was estimated according to the

potassium periodate method as described by Hartman (1953). The crude glycerol used in this study contained 38% of glycerol (0.38 g/g of crude glycerol). 2.63 and 5.26 g of crude glycerol were added to LPM to obtain the final 1% and 2% (w/v) of glycerol, respectively.

2.3.6. Statistical analysis

All statistical analysis was performed using the statistical package for social scientist (SPSS-PC 11.0 software, Chicago, Ill., USA). Empirical data are shown as the average from three independent experiments. One-way analysis of variance (ANOVA) was used for testing the differences between the treatments. P value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Lipase producing Dipodascus capitatus A4C

Low-cost substrates such as agro-industrial wastes, crude glycerol from biodiesel production process, and non-edible oils are now focused as the new carbon sources and inducers that may help in cost reduction and further development of the microbial lipase production. *Dipodascus capitatus* A4C was previously reported as a specific yeast strain that could produce lipases in both extracellular and cell-bound forms. Before low-cost carbon sources were investigated as promising low-cost substrates for lipase production by *D. capitatus* A4C, the lipase production medium was enriched with glucose as the main carbon source (LPM-G). However, the LPM-G showed considerable constitutive extracellular lipase (ECL) production of 2513 U/L after 72 h. Maximum cell biomass (CBM) production of 2.1 g/L was obtained after 72 h of cell cultivation in a fermentation medium at 30 ± 2 °C (Fig. 1). In addition, *D. capitatus* A4C also produced constitutive cell-bound lipase (CBL) with hydrolytic activity up to 620 U/L after 24 h.

3.2. Effect of nitrogen source

The requirement of nitrogen source varies with the type of microorganisms; some prefer organic nitrogen source and some other produce maximum enzyme in the presence of inorganic nitrogen source. Effect of several organic and inorganic nitrogen sources on cell growth and lipase production by *D. capitatus* A4C were investigated. These included organic nitrogen sources such as urea, peptone, and yeast extract and an inorganic nitrogen source (NH_4NO_3). The hydrolytic activities and cell biomass of the yeast strain were measured after 120 h of cell cultivation in a fermentation medium at 30 ± 2 °C. The yeast could grow well in LPM-G with yeast extract (g/L) but produced lower activity of ECL (U/L) and CBL (g/L) (Fig. 2). Urea and peptone showed a similar trend in CBM production of 0.8 (g/L) and 0.76 (g/L),

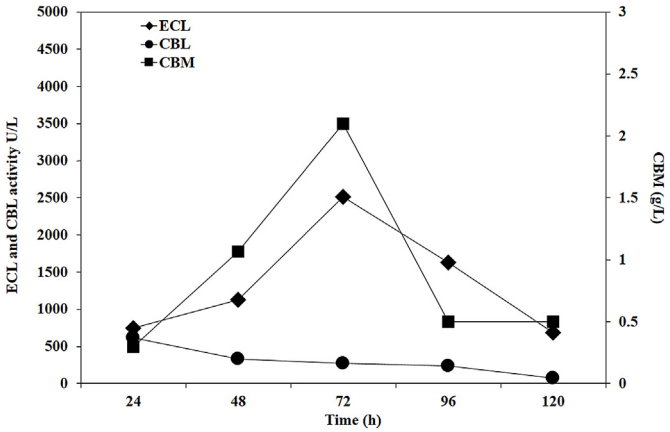

Fig. 1. Effects of glucose on lipolytic activity of extracellular (ECL) and cell-bound lipases (CBL), and cell biomass (CBM) production of *D. capitatus* A4C.

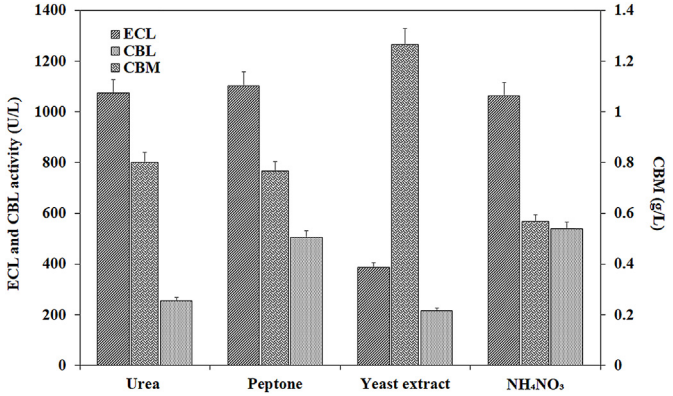

Fig. 2. Effects of nitrogen sources on lipolytic activity of extracellular (ECL) and cell-bound lipases (CBL), and cell biomass (CBM) production of *D. capitatus* A4C.

respectively, while minimum CBM production of 0.56 (g/L) was observed in LPM-G with ammonium nitrate. Interestingly, LPM-G with NH_4NO_3 revealed the highest CBL production of 539 U/L, followed by LPM-G with peptone and urea, which gave CBL production of 504 U/L and 254 U/L at 120 h, respectively. Peptone and urea showed ECL production as high as 1102 U/L and 1073 U/L, respectively (Fig. 2).

3.3. Combination of carbon source and inducer

Using non-edible oils or second-generation oils as carbon sources and/or inducers can help to reduce the production cost of lipase. The non-edible *Jatropha curcas* oil is one of the most promising and economical oil source for the production of cost-effective lipases. As it is a non-edible oil source, it does not affect the food prices and shares no competition with other edible oil sources in the food market. Lipase production media added either with *J. curcas* oil (LPM-JCO) and palm oil (LPM-PO) as a sole carbon source or combined with glucose (LPM-G-JCO and LPM-G-PO) as co-carbon sources were studied. Their effects on extracellular and cell-bound lipases, and cell biomass production were also investigated.

3.3.1. Effect on biomass production

The biomass production depends on not only the carbon source but also the coordinative performance of both carbon and nitrogen sources. It seems that LPM supplemented with *J. curcas* oil either as a sole carbon source or combined with glucose showed higher level of CBM production than LPM supplemented with palm oil. *J. curcas* oil could produce the highest level of CBM (7.6 g/L) when used as a single carbon source, whereas CBM of 5.7 was attained when mixed with glucose (LPM-G-JCO). While LPM supplemented with palm oil as a sole carbon source revealed 5.6 g/L of CBM production and 5.3 g/L of CBM was observed when palm oil was mixed with glucose (LPM-G-PO) (Table 1). However, the use of glucose as a sole carbon source in LPM gave rather lower CBM production as compared to its usage in mixed carbon

Table 1
Biomass production of *D. capitatus* A4C after supplementation of locally available low-cost substrates in lipase production medium (LPM).

LPM	Biomass (g/L)	Time (h)
LPM-JCO	7.6	72
LPM-G-JCO	5.7	72
LPM-PO	5.6	120
LPM-G-PO	5.3	24
LPM-M	1.7	120
LPM-M-JCO	5.0	120
LPM-CG	4.0	96
LPM-CG-JCO	7.0	120

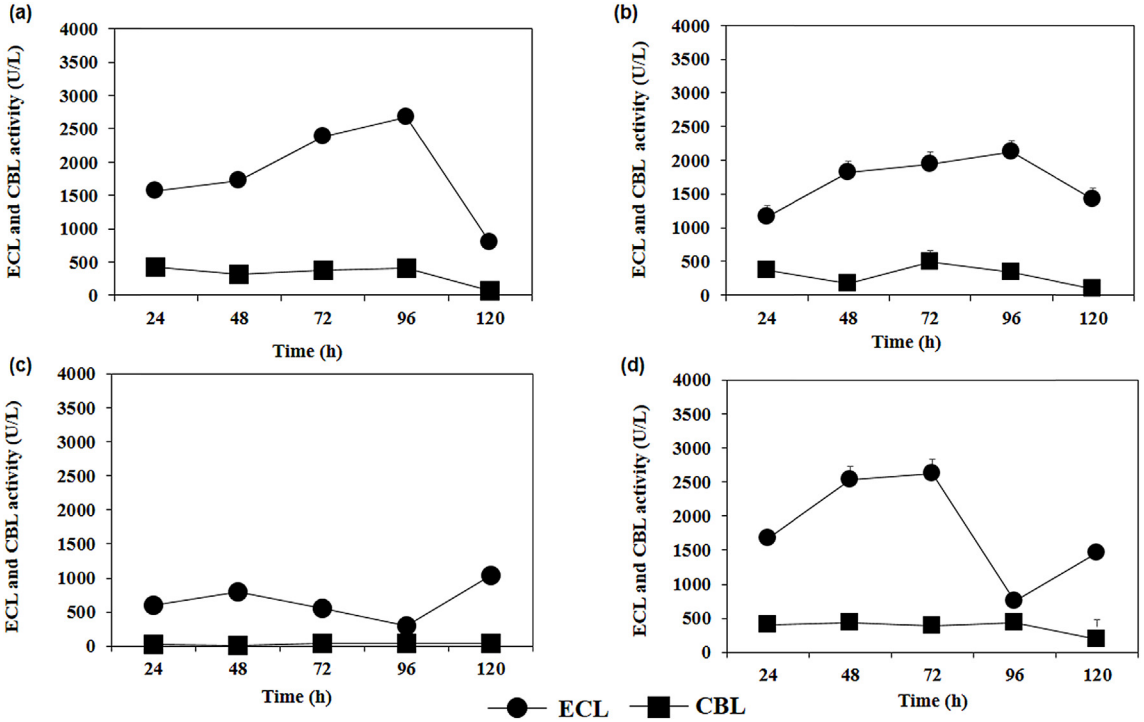

Fig. 3. Effects of *J. curcas* oil (LPM-JCO; a), glucose and *J. curcas* oil (LPM-G-JCO; b), palm oil (LPM-PO; c), and glucose and palm oil (LPM-G-PO; d) on hydrolytic performance of extracellular (ECL) and cell-bound lipases (CBL) of *D. capitatus* A4C.

sources (Fig. 1).

3.3.2. Effect on extracellular lipase production

The production of extracellular *D. capitatus* A4C lipase (ECL) was observed while cultivating the yeast strain in LPM containing single and mixed carbon sources including LPM-JCO, LPM-PO, LPM-G-JCO, and LPM-G-PO. When cultivated in LPM-JCO, *D. capitatus* A4C showed high ECL activity of 2670 U/L at 96 h followed by LPM-G-PO and LPM-G-JCO with ECL activities of 2627 U/L at 72 h and 2118 U/L at 96 h, respectively. The ECL production was observed in the following decreasing order LPM-JCO > LPM-G-PO > LPM-G-JCO > LPM-PO (Fig. 3).

3.3.3. Effect on cell-bound lipase production

Initially cell-bound lipase (CBL) was produced by *D. capitatus* A4C to degrade the available fatty substances. The yeast strain could produce CBL when grew in LPM-G-JCO, LPM-G-PO, and LPM-JCO in the range of 482.2 U/L at 72 h, 440.8 U/L at 48 h, and 429.5 U/L at 24 h, respectively. *D. capitatus* A4C revealed lower CBL production after 120 h of cell fermentation in LPM-PO (Fig. 3). Application of double carbon sources to the fermentation process causes an elongation to the mid and late exponential phases. The elongation in the exponential phase leads to an increase in the bioproduction process. The CBL produced in media containing glucose with a co-carbon source like LPM-G-JCO and LPM-G-PO performed better lipase production than without glucose like LPM-JCO and LPM-PO. The CBL production decreased in the following order LPM-G-JCO > LPM-G-PO > LPM-JCO > LPM-PO (Fig. 3).

3.4. Use of low-cost substrate as carbon source

Crude glycerol and molasses were screened as single and co-carbon sources for ECL, CBL and CBM production by *D. capitaus* A4C. The results are shown in Fig. 4 and Table 1. ECL production using glucose as main carbon source dropped down after 72 h, while it kept increasing until reached to the maximum level at 120 h when molasses was applied as a single carbon source. However, ECL, CBL, and CBM production with molasses (LPM-M) as a single carbon source showed similar behavior like glucose (LPM-G) as a single carbon source, (Figs. 1 and 4a).

3.4.1. Effect on biomass production

D. capitatus A4C grew well in LPM-CG-JCO and showed maximal biomass production of 7 g/L at 120 h followed by LPM-M-JCO (5 g/L) at 120 h, LPM-CG (4 g/L) at 96 h, and LPM-M (1.7 g/L) at 120 h. The mixed carbon sources gave higher growth than the single carbon source. Moreover, the mixed carbon sources not only produced higher CBM but also stimulated the production of lipases (Table 1 and Fig. 4).

3.4.2. Effect on extracellular lipase production

The ECL production was investigated using locally available low-cost carbon sources. Interestingly, the ECL produced in the LPM-CG-JCO fermentative medium performed the hydrolysis activity of palm oil better than LPM-M-JCO, LPM-CG, and LPM-M. The ECL activity of 1475 U/L was observed in LPM-CG-JCO after 120 h followed by LPM-M (1140 U/L) at 120 h, LPM-M-JCO (1082 U/L) at 24 h, and LPM-CG (1050 U/L) at 48 h (Fig. 4). However, the ECL production in LPM supplemented with low-cost substrates showed a decreasing tendency in the following order LPM-CG-JCO > LPM-M-JCO > LPM-CG.

3.4.3. Effect on cell-bound lipase production

The trend of CBL production using industrial wastes as low-cost substrates were quite similar with other treatments done with glucose, *J. curcas* oil, and palm oil. LPM-M showed higher CBL production of 373 U/L at 48 h followed by LPM-M-JCO (367 U/L) at 48 h, respectively. While LPM-CG and LPM-CG-JCO produced lower CBL activity of 305 U/L and 295 U/L at 96 h, respectively (Fig. 4).

3.5. The constitutive lipase production by D. capitatus A4C

The constitutive lipase production was investigated. Figs. 1 and 4a showed noticeable *D. capitatus* A4C constitutive lipase production both in extracellular and cell-bound forms. When 2% glucose or 2% molasses

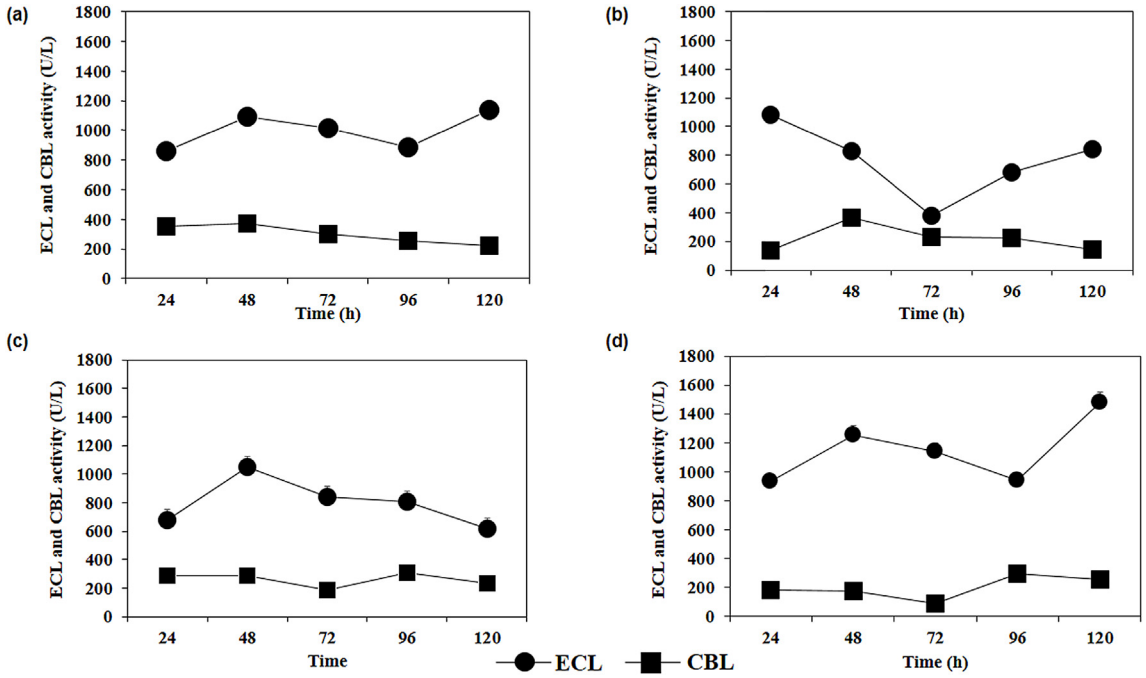

Fig. 4. Effects of molasses (LPM-M; a), molasses and *J. curcas* oil (LPM-M-JCO; b), crude glycerol (LPM-CG; c), and crude glycerol and *J. curcas* oil (LPM-CG-JCO; d) on hydrolytic performance of extracellular (ECL) and cell-bound lipases (CBL) of *D. capitatus* A4C.

was applied as a sole carbon source in LPM, the yeast strain could produce significant lipases in both ECL (2513 U/L at 72 h; LPM-G and 1140 U/L at 120 h; LPM-M) and CBL (620 U/L at 24 h; LPM-G and 373 U/L at 48 h; LPM-M) forms, respectively.

4. Discussion

Prior to applying the low-cost substrates as carbon sources, the yeast strain was cultivated using glucose as either sole or mixed carbon sources so that it can adapt the alteration in its nutrition. The cell growth and the lipases production in both ECL and CBL forms in the medium merely containing glucose were rather high (Fig. 1). The results were in agreement with Boonchaidung and Papone (2013) who found that *Candida* sp. KKU-PH2-15 could grow and produce lipase without lipidic carbon sources at 1.045 and 0.984 U/mL, with glucose and xylose as main carbon sources, respectively. Similar results of lipase production in the media containing non-lipidic carbon sources have been reported as constitutive lipase production by Nunes et al. (2014), De Almeida et al. (2013) and Colin et al. (2011). Not only ECL was produced constitutively by *D. capitatus* A4C but also the level of constitutive CBL production was rather high (Figs. 1 and 4a). Such a high level of constitutive lipase production may have been caused because of the habitat change, the yeast strains from rich fatty components habitat were shifted to an environment rich in glucose but the response of the strains toward the lipase production may have not changed. Similar results of CBL production by *Rhizopus oryzae* have also been reported (Musani et al., 1993). Dalmau et al. (2000) and Prabhakar et al. (2002) studied the effect of various carbon sources on the lipase production by *Candida rugosa*, *Aspergillus niger, Aspergillus flavus*, and *Aspergillus japonicus* and found that carbohydrate carbon sources were suitable for both cell growth and lipase production whereas lipidic sources were suitable only for inducing lipase production. Compared to inorganic nitrogen sources, organic nitrogen sources are good substrates for cell growth and biomass production. These nitrogen sources contain amino acids and peptides, water-soluble vitamins and carbohydrates (Castro et al., 2016), whereas inorganic nitrogen sources contain nutrients that satisfy only the minimal biomass growth requirements (Usall et al., 2002). The high cell growth of the *D. capitatus* A4C (Fig. 2) with the polypeptide nitrogen sources may have been caused due to the additional nutrients as well as the high nitrogen concentration present in the organic nitrogen sources. Whereas the low lipase production in the medium containing yeast extract would, probably was due to the undesired protein production in the highly rich medium with yeast extract, as reported previously (Ali et al., 2010; Burkert et al., 2004).

Lipase production is a costly process, mainly due to the cost of carbon sources used in the process. Different edible triacylglycerides i.e. olive oil, palm oil, sunflower oil, and soybean oil have been reported as carbon sources for yeast lipase production (Dominguez et al., 2003; Kumar et al., 2012). However, the use of edible oil for lipase production likely causes an increase of the food price (Pourzolfaghar et al., 2016). To reduce the cost of the lipase production, the use of low-cost carbon sources can be a promising strategy (Boonchaidung and Papone, 2013). Various low-cost substrates, such as molasses (Gutarra et al., 2009; Soleymani et al., 2017), glycerol (De Almeida et al., 2013; Fabiszewska et al., 2015), olive pomace, and wheat bran (Lu et al., 2017) have been reported for lipase production. However, none of these studies reported about the use of single lipidic, mixed lipidic, and non-lipidic carbon sources to investigate the constitutive lipase production.

When grown in the medium supplemented with non-edible *J. curcas* oil (LPM-JCO); containing unsaturated oleic acid 44.7% and polyunsaturated linoleic acid 32.8% (Akbar et al., 2009), *D. capitatus* A4C showed higher CBM as well as lipase production (Fig. 3a), may possibly due to the substrate specificity for the hydrolysis. The stability of CBL activity in the media containing co-carbon sources was higher than the media containing single carbon sources (Fig. 3). The synergistic effect of the co-carbon sources was also observed in cell-bound lipase production by *Rhizopus oryzae* (Arnau et al., 2010). Two types of cell-bound lipases are localized into the *Rhizopus oryzae* IFO 4697 cells. One into the space and can easily be released into the medium and the other is firmly bound to the cell membrane and that could only solubilize when treated with the detergent (Hama et al., 2006). In the presence of gum arabic which would have facilitated more release of the cell-bound and intracellular lipases into the medium (Bresciani et al., 2014). Thus, the ECL activity of *D. capitatus* A4C increased whereas the CBL activity decreased with the increase in fermentation time. The CBL dominated over the ECL at the early fermentation hours (Figs. 1, 2 and 4). On the

other hand, in the presence of glucose both CBL and ECL production started at the early fermentation time (Figs. 1, 3b, 3d, 4a and 4b) indicating that in the presence of glucose yeast cells could adapt themselves better. The energy received from glucose might help the cells to be ready for both cell growth and biochemical production as the lipase production is an entirely growth dependent process. The elongation of exponential growth phase increases the bioprocess lifespan and thus enhancing the production of the bioproducts (Huang et al., 2012). When molasses and glucose were applied to the lipase production medium, the CBL production was rather high in the early hours (Figs. 1 and 4a). Additionally, in the presence of co-substrates the ECL production was more induced than CBL production (Figs. 3b, 3d and 4b, 4d). Louhasakul et al. (2016) reported lipid and cell-bound lipase production from different strains of *Yarrowia lipolytica* using palm oil mill effluent (POME) as carbon source. Highest cell-bound lipase activity of 610 ± 87 U/L has been reported, however using molasses as the non-lipidic carbon source for cell-bound lipase production has not been reported before.

Among the reported constitutive lipase producing yeasts such as *Yarrowia lipolytica* (Nunes et al., 2014) and *Candida rugosa* (Lotti et al., 1998), *D. capitatus* A4C produced significantly higher ECL and CBL activities. The molecular approach for the optimization of the constitutive lipase production has been reported by Wang et al. (2012), who cloned *Yarrowia lipolytica* lipase *LIP2* gene into a constitutive expression vector to enhance constitutive lipase production and reported 13500 U/mL of ECL activity. However, the optimization of the bioprocess system for the constitutive lipase production that has not yet been reported can be a promising trend to produce cheaper lipases by using the low-cost carbohydrate carbon sources.

5. Conclusion

This study has shown the possible cultivation of a newly isolated yeast *D. capitatus* A4C in media, based on inexpensive, locally available low-cost substrates for extracellular and cell-bound lipases production. The medium containing 2% *J. curcas* oil was the most suitable medium for extracellular lipase production, whereas the medium containing 2% glucose or 2% molasses was more suitable for the production of constitutive lipase expressed as cell-bound form. The use of suitable low-cost carbohydrate carbon sources and inducers may contribute greatly to the effective manipulation of extracellular and cell-bound lipases production.

Conflicts of interest

The authors declare no conflicts of interest.

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