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Alamat (*Address*)
Pusat Penelitian Biologi-LIPI
Kompleks Cibinong Science Center (CSC-LIPI)
Jalan Raya Jakarta-Bogor KM 46,
Cibinong 16911, Bogor-Indonesia
Telepon (021) 8765066 - 8765067
Faksimili (021) 8765059
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id
jurnalberitabiologi@gmail.com

Keterangan foto cover depan: Studi perbanyakan vegetatif pada bidara upas koleksi Kebun Raya Bogor, sesuai dengan halaman 169
(*Notes of cover picture*): (*Study of vegetative propagation on bidara upas of bogor botanical garden collection, (as in page 169)*

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CO-CULTURE OF AMYLOLYTIC FUNGI *Aspergillus niger* AND OLEAGINOUS YEAST *Candida orthopsilosis* ON CASSAVA WASTE FOR LIPID ACCUMULATION [Akumulasi Lipid Oleh Kultur Campuran Kapang *Aspergillus niger* dan Khamir *Candida orthopsilosis* Pada Media Limbah Singkong]

Atit Kanti ✉ and I Made Sudiana
Research Center for Biology-LIPI
Jl. Raya Bogor Km 46, CSC, Cibinong 16911, Bogor, Indonesia
email: atityeast@gmail.com

ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi efektivitas kultur campuran antara fungi (*A. niger*) amilolitik dan khamir pengakumulasi lipid (*Candida orthopsilosis*) dalam produksi total lipid, dengan menggunakan substrat limbah singkong. Sepuluh isolat terseleksi dapat tumbuh pada media yang mengandung 5 % pati, dimana isolat yang mampu tumbuh paling baik adalah *A. niger*, sedangkan mikroba yang lain tumbuh lebih lambat. *Saccharomycopsis fibuliger* mempunyai kemampuan amilolitik lebih rendah dibandingkan dengan *A. niger*. Kedua isolat tersebut dipilih untuk penelitian akumulasi lipid. Aktivitas amilolitik dipengaruhi oleh suhu, pH dan sumber nitrogen. Suhu dan pH optimum untuk produksi enzim amilase adalah 30 °C dan pH 7.0. Yeast ekstrak dan sodium nitrat merupakan sumber nitrogen yang baik untuk produksi amilase. Kultur campuran fungi (*A. niger*) dan khamir (*C. orthopsilosis*) yang ditumbuhkan pada limbah singkong memberikan hasil yang optimum untuk produksi biomassa dengan kandungan lipid tinggi. Jenis lipid yang dihasilkan didominasi oleh asam oleat dan asam stearat. Kultur campuran *A spergillus niger* dan khamir pengakumulasi lipid, *C. orthopsilosis,* yang mempunyai kemampuan untuk mengakumulasi lipid pada media limbah singkong menunjukkan bahwa mikroba tersebut merupakan mikroba potensial untuk produksi *biofuel*.

Kata kunci: *Aspergillus niger*, *Candida orthopsilosis*, kultur campuran, khamir pengakumulasi lipid

ABSTRACT

The objective of this study was to evaluate co-culture effectivity of amylolytic fungi *Aspergillus niger* and lipid accumulating yeast, *Candida orthopsilosis,* for lipid accumulation on cassava waste. When grown in 5 % starch medium, ten selected isolates were able to grow, but best growth was observed on *Aspergillus niger*, other microbes grew slower. Moderate growth was observed on *Saccharomycopsis fibuliger*. Due to their superiority on starch hydrolyzes and lipid accumulation, both isolates were then selected for further studies. *Aspergillus niger* and *S. fibuliger* were amylolytic microbes. The amylolytic activities were affected by temperature, pH and nitrogen sources. Optimum temperature and pH for enzyme production were 30°C and 7.0 respectively. Both yeast extract and sodium nitrate were good nitrogen sources for amylase production. On cassava waste, the highest biomass and total lipid content were obtained by co-culture of *Aspergillus niger* and lipid accumulating yeast *C. orthopsilosis.* Major lipid composition was oleic acids and strearic acids. The ability of co-culture of *A. niger* and lipid accumulating yeast *C. orthopsilosis* grew and accumulated lipid on cassava waste would suggest that these culture were potential candidate for biofuel production.

Key words: *Aspergillus niger*, *Candida orthopsilosis,* co-culture, lipid accumulating yeast

INTRODUCTION

Concern on petroleum reserve and its environmental impact trigger the search for renewable energy resources (Arroyo and Galiana, 2005; Syakti *et al*., 2013). Microbial sources for biofuel production have gained many attentions (Datar *et al*., 2004; Singh and Harvey, 2010; Sudiana *et al*., 2014). Scientist are to explore the possibility of generating biofuel from oleaginous yeast (Johnson *et al*., 2011). However the viable economic biofuel production has yet under intensive investigation (Abila, 2015). Most of the production cost is spent for fed material Agricultural waste which mostly contained lignocellulose is renewable for biofuel production (Santa-Maria *et al*., 2013). Cassava (*Manihot esculenta* Crantz) pulp is the solid waste produced by starch production. This pulp contains starch content (50-60% dry basis), causing an environmental problem with disposal. On the other hand, cassava waste is potential source for ethanol production (Srinorakutara and Kaewvimol, 2006).

The utilization of this waste for biodiesel production will enhance its value. The utilization of cassava waste for biodiesel at least require 3 steps that include: saccharification by amylolytic and cellulolytic microbes to produce assimilable carbon sources (Chen *et al*., 2012); lipogenesis by which carbon is absorbed and accumulated into neutral lipid, mainly triacylglycerol (Kanti *et al*., 2013); and trasmethylation (transesteriphication) of neutral lipid into biodiesel (Meng *et al*., 2009).

Previous works on searching potential amylolytic microbes have been conducted by many scientists (Chen *et al*., 2012; Ouagadougou and Faso, 2012; Vihinen and Mäntsälä, 1989). Filamentous fungi are important microbe due to their high potential of en-

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zymes production. Many enzymes produced by fungi related to biotechnological applications in several industrial sectors. *Aspergilus, Penicillium* and *Trichoderma* produce amylase. All isolates exhibited enzymatic potential. *Penicillium granulatum* (FCBP1080), *Aspergillus raperi* (FCBP1007) and *Aspergillus speluneus* (FCBP1128) were hyper active in starch medium and showed the increased growth in starch medium (Khokhar*et al*., 2011).

To obtain higher amylase production, fermentation technique, which include submerge and solid state fermentation were introduced. Under solid state fermentation, *Rhizopus microsporus* var. *rhizopodiformis* produce high alpha amylase on various substrates which include wheat bran, cassava flour, sugar cane bagasse, rice straw, corn cob and crushed corn cob. Initial moisture content was adjusted through humidification with distilled water, tap water, or saline solutions Segato Rizzatti (SR), Khanna or Vogel. The best substrate for amylase production was wheat bran with SR saline solution (1:2 v/v) (Peixoto-Nogueira *et al*., 2008).

In addition to fermentation technique, amylolytic activities are affected by multi factors which include pH, temperature, initial moisture content, particle size, and the presence of enzyme production stimulator and growth inhibitor (Nguyen *et al*., 2000).

The strategy to increase lipid accumulation can be carried out by increasing production of monomeric sugar by amylolytic microbes. The excessive fermentable sugar product was then accumulated by oleaginous microbes for intracellular lipid accumulation. Polysaccharide substances including starch are hydrolyzed by amylase which mostly produced by amylolytic fungi. The enzymes of amylolytic fungi have been extensively exploited in diverse ethanol production. Due to the importance of amylolytic fungi in industrial processes, the genes responsible for amylase production were verified. Amylolytic genes of 85 strains of fungi from the phyla *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Zygomycota* were annotated on the genomic scale according to the classification of glycoside hydrolase (GH) from the Carbohydrate-Active enzymes (CAZy) database (Levasseur *et al*., 2013). Comparisons of gene abundance in the fungi suggested amylolytic genes arrangement are responded to their respective lifestyles (Battaglia *et al*., 2011). Amylolytic enzymes in family GH13 were divided into four distinct clades identified as heterologous α-amylases, eukaryotic α-amylases, bacterial and fungal α-amylases and GH13 α-glucosidases (Chen *et al*., 2012).

Aspergillus niger is other popular amylase producer (Abouzied and Reddy, 1986; Latorre-García *et al*., 2005). Several amylolytic yeast have been used in industrial processes (Ouagadougou and Faso, 2012). In addition to fungi and yeast, bacteria including *Bacillus licheniformis* (Ruiz *et al*., 2011) and *Lactobacillus plantarum* (Giraud *et al*., 1991) produced amylase as well. These data showed that amylolytic microbes are quite divers (Chen *et al*., 2012; Horn *et al*., 1988).

In general, cassava starch is commonly used for amylase production. Almost all amylolytic microbes are effective to hydrolyze starch. Starch from cassava is solid waste, which should not be disposed into water body due to its environmental impact. Therefore, utilizing cassava waste for lipid production has double benefit. This present study described the importance of co-culture amylolytic fungi and oleaginous yeast for lipid production.

MATERIALS AND METHODS

Microbial sources

We isolated ten isolates of yeasts and fungi from Bali and Sulawesi. These isolates were maintained in collection of Microbial Biosystematic Laboratory, Research Center for Biology-LIPI, and used for this study.

rDNA sequence determination.

Total DNA template was extracted from freshly -grown cells on the Potato Dextrose Broth (Butinar *et al*., 2005). PCR amplification of the partial Internal Transcribed Spacer (ITS) ribosomal subunit with primers ITS 4: 5`– TCC TCC GCT TAT TGA TAT GC – 3`and Primer ITS 5: 5`– GGA AGT AAA AGT CGT AAC AAG G –3`(White *et al.,* 1990; O`Donnell, 1993) was performed using GoTaq master mix (Promega, M7122). PCR products were visualized on 2% agarose and sequenced with both primers using Big Dye terminator v3.1. Cycle Sequencing Ready Reaction Kit (Applied Biosystems)

following the manufacturer's instructions. The partial 26S sequences determined in this study were compared to those in the EMBL/GenBank/DDBJ databases using the nucleotide Basic Local Alignment Search Tool (BLASTn) (Altschul *et al.*, 1997).

Screening of amylolytic microbes

Ten isolates of fungi and yeast were grown in PDA for 72 h, at 30 °C. The culture was then transferred to minimal medium containing 1% starch as carbon sources and 0.1 % yeast extract as N-sources. After growing on the second medium, the plates were refrigerated at 4 °C (1 to 2 days) to convert dissolved starch into an insoluble and highly turbid suspension within the agar. The amylolytic isolates exhibited clear zones around the colonies. Yeast colonies and fungi showing a clear zone diameter that was 1.3 to 4.4 times greater than colony size (ranging from 3 to 21 mm) were tested for their abilities to ferment starch using the inverted Durham tube method.

Determination of amylolytic activity

The extracellular amylolytic activity that was occured during the fermentation of starch by the amylolytic yeasts and fungi was determined by measuring the reducing sugar by a colorimetric method, based on the reduction of 3,5-dinitrosalicylic acid. Each reaction mixture which contained 0.1 mM sodium acetate acetic acid buffer (pH 5.0), 4.6 mM sodium chloride, and 4.5 mg of Lintner starch per ml in a final volume of 2.2 ml, was incubated for 60 min at 45 °C. Supernatant obtained by centrifugation of yeast cultures were used as the samples.

The reaction was terminated by adding 2 ml of 3,5-dinitrosalicylic reagent. The reducing sugar was determined colorimetrically at 546 nm (UV mini 1240, Shimadzu), as described by Liao *et al*. (2010). One unit of enzyme activity was defined as the amount that liberated 1 µmol of reducing sugar group per min per ml of enzyme sample. A standard curve for the colorimetric assay was constructed by using maltose as the reducing sugar.

Evaluation of yeast strains for oleaginous properties

Determination of oleaginous yeast, purified strains were initially streaked onto YM plates and grown for 2 days at 28 °C. Fresh colonies were then inoculated into 250-mL Erlenmeyer flasks containing 50 mL modified medium containing (in g/L): glucose 20, $(NH_4)_2SO_4$ 5, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5, and yeast extract 0.5 (Pan *et al.*, 2009) and grown using rotary shaker at 180 rpm, 28 °C, for 2 days. The culture was then used as inoculum. Five millilitre of inoculum were transferred to 45 mL (in a 250-mL Erlenmeyer flask) of screening medium containing nitrogen-limited medium (in g/L): glucose 40, $(NH_4)_2SO_4$ 2, KH_2PO_4 2, NaH_2PO_4 2, $MgSO_4 \cdot 7H_2O$ 1.5 and yeast extract 0.5, supplemented with a 100-fold diluted trace element solution (Wu *et al.*, 2010). The trace element solution contained (in g/L): $CaCl_2.2H_2O$ 4.0, $FeSO_4.7H_2O$ 0.55, citric acid H_2O 0.52, $ZnSO_4.7H_2O$ 0.10, $MnSO_4.H_2O$ 0.076, and 100 µl of 18M H_2SO_4. The medium was sterilized at 121 °C for 15 min (Wu *et al.*,2011). The culture was incubated on a rotary shaker at 180 rpm and 28 °C for 5 days. The total lipid of the cultures was determined following previously described methods by Sitepu *et al.* (2012).

Growth and fermentation procedure

All growth and fermentation experiments were carried out at 25 and 30 °C. Stock cultures were grown in YPD slants. Firstly, to get enough biomass, the cultures were grown in 1 % (w/v) medium. In brief, one loopful culture from a fresh slant was inoculated into 6 ml of YPD medium in a test tube and incubated for 24 h. Secondly, after 24 hours, the cultures were mixed with 150 ml of media contained 10 % (w/v) cassava waste starch in a 500-ml Erlenmeyer flask. The medium was incubated on a rotary shaker (Bioshaker BR 300LF, TAITEC, Japan) at 125 rpm. The biomass and lipid content were determined.

CFU determination of fungi and yeast

The biomass of fungi and yeast was determined according Kanti *et al.* (2013). One mililitre of samples was poured in PDA incubated at 30 °C for 72 h. The number of colonies were then counted. Biomass of fungi and yeast was expressed as CFU/ml.

Lipid composition analysis

The total lipid concentration was determined by gas chromatographic analysis of the total fatty acids directly transmethylesterified from dried cell (Kumon *et al.*, 2002). Cells Kumon *et al.*, 2002 Kumon, Y., Yokochi, T., Nakahara, T., Yamaoka, M., Mito, K., 2002. Production of long-chain polyunsaturated fatty acids by monoxenic growth of labyrinthulids on oil-dispersed agar medium, 60, 275–280. One milliliter of 10% methanolic HCl and 0.5 ml methylene chloride were added to the dried biomass cell sand incubated at 60 °C for 3 h for direct methyl esterification. The reaction was terminated by the addition of 2 ml saturated NaCl solution and 1 ml hexane. The resultant methyl esters recovered in the hexane layer were then applied to a gas chromatograph (GCMS-QP 2010-Ultra; Shimadzu, Kyoto, Japan) equipped with a FAMEWAX capillary column (30 m×0.25 mm i.d., GL Science, Tokyo, Japan) under temperature programming (150–250 °C at 5 °C/min increments). Peanut oil (Nacalai Tesque, Kyoto, Japan) was transmethylesterified and used as the reference material.

RESULTS

Amylolytic microbes

When grown in 5% starch medium, all microbes was able to grow. Best growth was observed on *A. niger* (Table 1), meanwhile, other microbes grew slower. Moderate growth was observed on *Saccharomycopsis fibuliger*. Clear zone surrounding growing colonies was observed after addition of iodium, indicating starch was hydrolyzed by microbes, produced reducing sugar. The highest amylolytic activities was observed on *A. niger*, subsequently followed by *S. fibuliger* (Table 1). The two isolates were then selected for further studies.

Effect of temperature on biomass growth on starch

Temperature affected the growth of both *Aspergillus niger* and *S. fibuliger* (Figure 1). Better growth was observed at 30 °C than 25 °C. *A. niger* used easily starch compare to *S. fibuliger*. Maximum growth was observed after 78 hours incubation.

Effect of temperature on amylase activities

Likewise growth profile, activity of amylase was affected by temperature. Higher activity was occurred at 30℃.

Effect of pH on amylase activities

The highest amylase activity was obtained at pH 7. Maximum amylase activity (15.2 Unit) was achieved after 96 h fermentation (Figure 3). Enzyme production at pH 7 was 15 % greater than at pH 6 and 8.

Table 1. Amylolytic ability of microorganisms grown on 1 % starch at 30 °C for 72 hour (*Kemampuan amilolitik mikroba yang ditumbuhkan pada medium 1 % pati, suhu 30 °C selama 72 jam*)

No.	Isolates (Isolat)	Amylolytic ratio *(rasio amilolitik)*	Growth on starch *(pertumbuhan pada pati)*[a]	Lipid accumulation *(akumulasi lipid)*[b]
1	*Aspergillus niger*	4.3	+++	23.1
2	*Saccharomycopsis fibuliger*	2.3	++	11.2
3	*Candida orthopsilosis*	1.8	+	25.6
4	*Rhizopus oligosporus*	1.9	++	11.1
5	*Mucor circinoides*	1.9	+	8.9
6	*Saccharomyces cerevisiae*	1.5	+	6.8
7	*Neurospora crassa*	1.9	+	7.2
8	*Neurospora sitophyla*	2.1	+	6.8
9	*Trichoderma viride*	1.9	+	6.3
10	*Penicillium* sp	1.9	+	6.7

Note (*Keterangan*): [a]+++, best growth (*tumbuh sangat baik*), ++ good growth (*tumbuh baik*), + fair growth (*tumbuh*). [b]% Cell dry weight (*berat kering sel*)

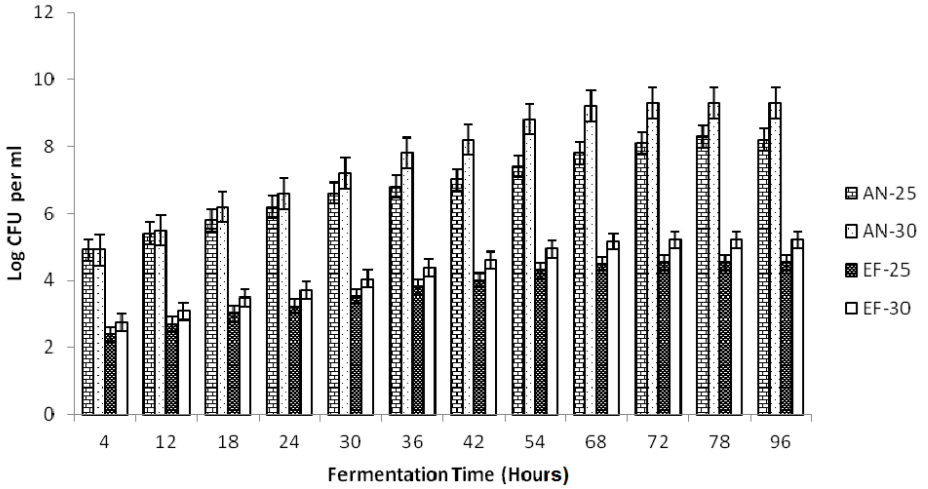

Figure 1. Biomass growth of *Aspergillus niger* (AN) and *Saccharomycopsis fibuliger* (EF) grown in 5 % starch under shaking condition (125 rpm) at 25 and 30 °C. Bar indicate *sd*, with n=5 (*Pertumbuhan biomassa Aspergillus niger (AN) dan Saccharomycosis fibuliger (EF) yang ditumbuhkan pada 5 % pati kecepatan shaker (125 rpm) pada suhu 25 °C dan 30 °C. Garis mengindikasikan sd, dengan n=5*)

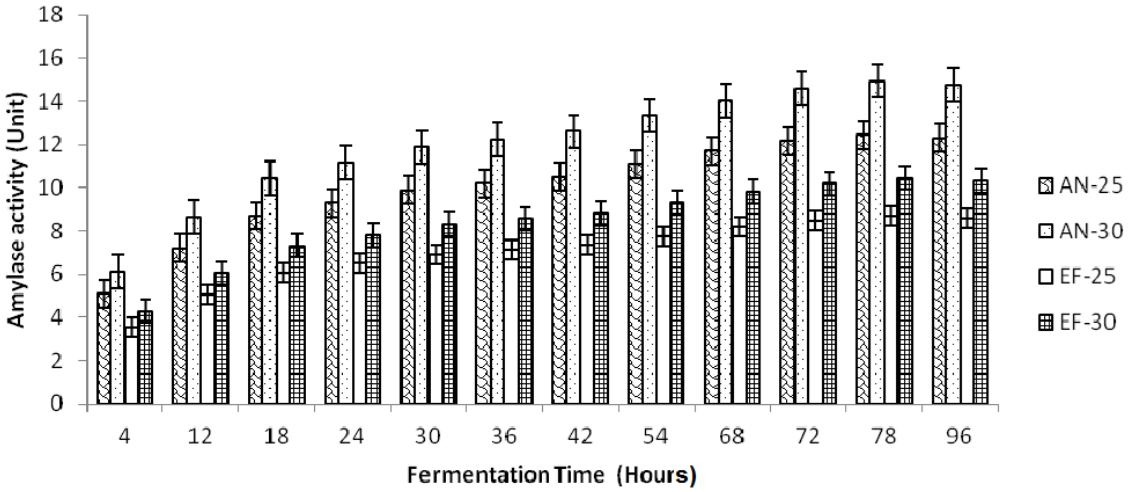

Figure 2. Effect of temperature on *Aspergillus niger* (AN) and *Saccharomycopsis fibuliger* (EF) growth and amylase activity at 25 and 30 °C. Bar indicate *sd*, with n=5 (*Pengaruh temperatur terhadap kecepatan tumbuh Aspergillus niger (AN) dan Saccharomycopsis fibuliger (EF)dan aktivitas amilase pada suhu 25 dan 30 °C. Garis menunjukkan sd, dengan n=5*).

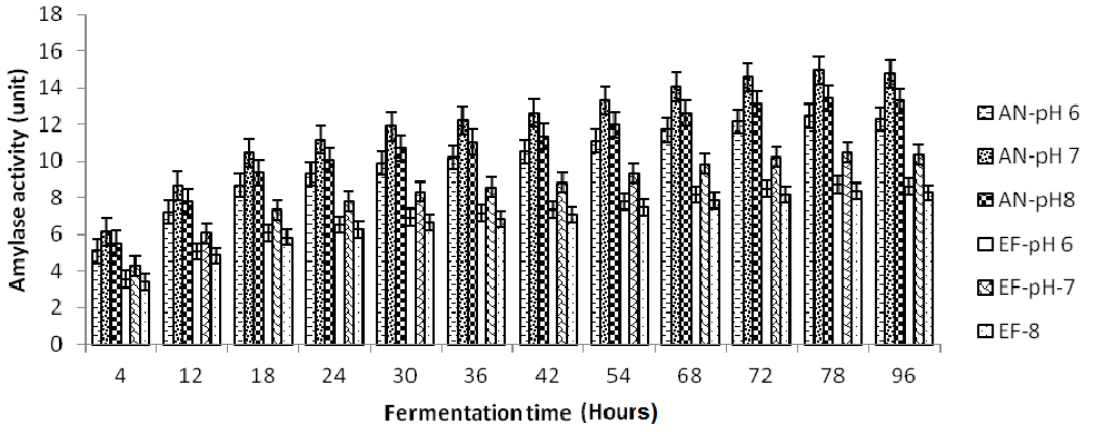

Figure 3. Effect of pH (6,7 and 8) on amylase activity of *Aspergillus niger* (AN) and *Saccharomycopsis fibuliger* (EF) grown on 10% (w/v) cassava waste starch under shaking condition (125 rpm), at 30 °C (*Pengaruh pH untuk pertumbuhan dan aktifitas amilase Aspergillus niger (AN) dan Saccharomycopsis fbuliger (EF) yang ditumbuhkan pada 10 % (w/v) limbah singkong pada kondisi aerasi (125 rpm), pada suhu 30° C*

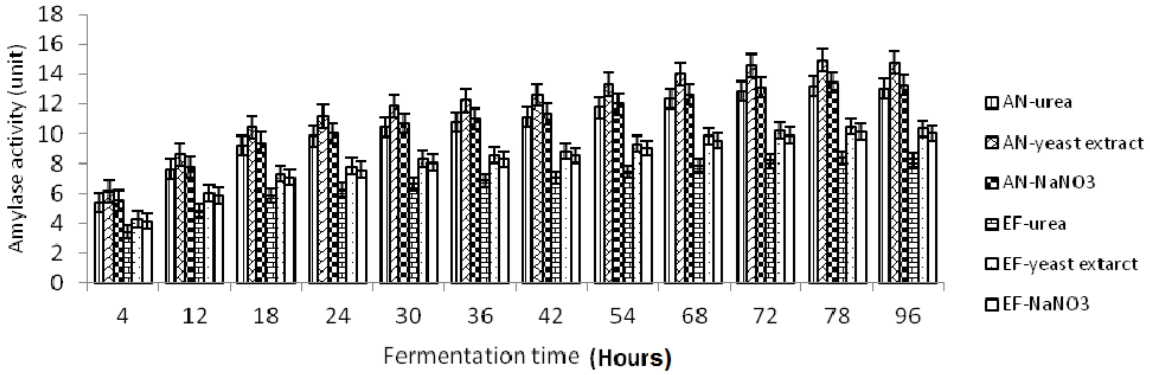

Figure 4. Effect of nitrogen sources on amylase production of *Aspergillus niger* (AN) and *Saccharomycopsis fibuliger* (EF) grown on 10 % (w/v) cassava waste starch under shaking condition (125 rpm), at 30 °C. Bar indicate *sd*, with n=5 (*Pengaruh sumber nitrogen untuk produksi amilase pada Aspergillus niger (AN) and Saccharomycopsis fibuliger (EF) yang ditumbuhkan pada 10% (w/v) limbah singkong pada kondisi aerasi (125rpm), pada suhu 30 °C. Garis menunjukkan sd, dengan n=5*).

Effect on nitrogen sources on amylase activities

Nitrogen sources affect the amylase production. In the case of *A. niger*, yeast extract was best nitrogen sources, however for *S. fibuliger* sodium nitrate and yeast extract were good nitrogen sources (Figure 4).

Oleaginous activities

Aspergillus niger has the highest amylolytic activity, meanwhile, *Candida orthosilopsis* produced the highest total lipid (Table 1). Therefore, the co-culture of both microbes was conducted. As expected, co-culture of amylolytic fungi (*A. niger*) and

oleaginous yeast (*C. orthosilopsis*) produced the highest neutral lipid (Table 2). Highest biomass was also observed on co-culture of *A. niger* + *C. orthopsilosis*

Fatty acid composition of oleaginous yeast

The fatty acid composition of neutral lipid mostly composed oleic acids and stearic acids (Table 3). Otherwise, fatty acid containing 14 carbon and 22 carbon were minor.

DISCUSSION

Several isolates were able to hydrolyze starch (Table 1) which suggests that fungi and yeast are potential for amylase production. *Aspergillus niger* and *Saccharomycopsis fibuliger* consistently hydrolyze starch both utilizing pure starch (Table 1) and cassava waste starch under various temperature, pH and nitrogen sources (Figure 1-4). The optimal temperature, and pH for growth and amylase synthesis might be different for amylolytic microorganism (De Mot *et al*., 1985; Ouagadougou and Faso, 2012). We observed that optimal condition for growth and amylase production on cassava waste starch was at 30 °C and pH 7,0 by shaking (Figure 1-4). Previous works exhibited that amylolytic activity was obtained at pH 5 to 8. (Abouzied and Reddy, 1986).

Aspergillus niger is one of the most important microorganisms used in industrial biotechnology, and generally consider as safe organism (Schuster *et al*., 2002). It has been used for many decades to produce extracellular (food) enzymes and citric acid. In fact, citric acid and many *A. niger* enzymes are considered Generally as Safe (GRAS) by the United States Food and Drug Administration. In addition, *A. niger* is used for biotransformations and waste treatment. In the last two decades, *A. niger* has been developed as an important transformation host to overexpress food enzymes (Vassilev *et al.*, 1997). Being pre-dated by older names, the name *A. niger* has been conserved for economical and information retrieval reasons and there is a taxonomical con-

Table 2. Total lipid content of microbes grown on cassava waste (*Kandungan lipida total mikroba yang ditumbuhkan pada limbah singkong*)

No.	Isolate (*Isolat mikroba*)	Total lipid* (%/DW) (*Lipida total*)	Biomass (g/L) (*Biomassa*)
1	*Aspergillus niger*	35,4 ± 3.6	8.8 ±2.1
2	*Saccharomycopsis fibuliger*	17,6 ± 2.9	3.9 ± 1.9
3	*Candida orthosilopsis*	25.6 ± 1.9	8.9 ±1.3
4	*Aspergilusniger + Candida orthopsilosis*	67,8 ± 4.2	9.4 ± 1.8

*Total lipid per cell dry weight of culture grown 7 days on cassava waste, under shaking 125 rpm, at 30 °C (*Lipida total per berat kering sel kultur mikroba yang ditumbuhkan pada limbah singkong selama 7 hari dengan kondisi aerasi (125 rpm) pada suhu 30 °C*).

Table 3. Fatty acid composition (*Komposisi asam lemak*)

Isolate (*Isolat*)	Relative fatty acid composition (wt/wt)(*Komposisi asam lemak*)								
	14:0	16:0 Palmitic	16:1	18:0 Stearic	18:1 Oleic	18:2	18:3	20:0	22:0
Aspergillus niger	1.3	18.9	6.9	17.2	38.4	11.3	1.6	1.9	1.2
Candida orthopsilosis	1.1	16.9	7.2	24.3	38.5	6.9	1.1	1.1	1.3
Aspergilus niger + Candida orthopsilosis	1.1	17.2	6.9	24.3	38.5	6.9	1.1	1.1	1.3
Saccharomycopsis fibuliger	1.1	16.7	7.4	24.3	38.5	6.9	1.1	1.3	1.1

sensus based on molecular data that the only other common species closely related to *A. niger* in the *Aspergillus* series *nigri* is *A. tubingensis*. Like other filamentous fungi, *A. niger*s should be treated carefully to avoid the formation of spore dust. However, compared with other filamentous fungi, it does not stand out as a particular problem concerning allergy or mycopathology (Schuster *et al*., 2002).

Aspergillus niger was also exploited to produce α-amylase for enzymatic hydrolysis of cassava waste starch for producing glucose syrups (Ruiz *et al*., 2011). They observed optimal enzyme reactions with 100 g of starch per L were: α-amylase at pH 5.0, 80 °C and enzyme dosage of 130.5 U g-1 of starch; and glucoamylase, pH 4.5, 70 °C and enzyme dosage of 81.5 U g-1 of starch. The glucose syrups were then fermented into alcohol by a wild strain of *Candida* sp. isolated from sugar cane juice, obtaining volumetric ethanol productivities around 1.8-3.2 g L-1 h-1. The amylase production optimum at 72 h, the same optimum fermentation time was also observed by Omemu *et al*. (2005) on cassava waste to produce glucose and maltose.

Nitrogen sources affect amylase production by *S. fibuliger* (Figure 4). Yeast extract and sodium nitrate was good nitrogen source for amylase production. Previous research on the amylolytic yeast *S. fibuligera* DSM-70554 grown on cassava starch with oxygen saturation (50% dissolved oxygen tension), temperature (34 °C) and pH (5.5) showed a significant influence on α-amylase and glucoamylase (González *et al*., 2008).

The production of amylase by *Aspergillus niger* on three cassava whey media in liquid shake culture was compared. The supplemented cassava whey (SCW) medium exhibited amylase activity of 495 U/ml. Biomass cropped was 1.63 g/l in the SCW medium (Pothiraj *et al*., 2006). Yeast extract employed as a nitrogen supplement increased biomass yield of *A. niger* to 2.75 g/l with maximum amylase activity of 643 U/ml. Sodium nitrate ($NaNO_3$) as nitrogen supplement had the lowest biomass yield of 0.77 g/l and amylase activity of 206 U/ml. Thus yeast extract as nitrogen supplement of cassava whey medium supported maximum production of amylase and biomass of *A. niger* (Oshoma *et al*., 2010).

Assimilable carbon sources was then converted into neutral lipid (Table 2). The lipid composition after esterification was mainly oleic acids and strearic acids (Table 3). Co-culture of *A. niger* and *C. parasilopis* produced highest lipid content (67.8 %).

In the same fashion, earlier work also observed that direct fermentation of unhydrolyzed potato starch to ethanol by co-culture of an amylolytic fungus *A. niger* and fermentative yeast *Saccharomyces cerevisiae* produced higher yield (Abouzied and Reddy, 1986). Amylolytic activity, rate and amount of starch utilization, and ethanol yields increased several-fold in co-culture versus the monoculture due to the synergistic metabolic interactions between the species. Ethanol yields were maximal when fermentations were conducted anaerobically.

CONCLUSION

Aspergillus niger and *Saccharomycopsis fibuliger* are amylolytic microbes. The ability of *A. niger* to hydrolyze starch was greater than *S. fibuliger*. Co-culture of an amylolytic fungi *A. niger* and oleaginous yeast *C. orthopsilosis* was effective for converting cassava starch waste into neutral lipid. The fatty acid composition was mainly oleic acids and stearic acids which implies that it can be used as fed material for biodiesel production.

ACKNOWLEDGEMENT

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Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput harus menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up to date*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan baru, agar dapat segera diketahui oleh umum. Artikel yang ditulis tidak lebih dari 10 halaman. Hasil dan pembahasan boleh digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah Bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul diberikan dalam bahasa Indonesia dan inggris. Judul harus singkat, jelas dan mencerminkan isi naskah dengan diikuti oleh nama serta alamat surat menyurat penulis dan alamat email. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*).
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam Bahasa Inggris merupakan terjemahan dari Bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Perlu disebutkan juga studi terdahulu yang pernah dilakukan terkait dengan penelitian yang dilakukan.
5. **Bahan dan cara kerja**
   Bahan dan cara kerja berisi informasi mengenai metode yang digunakan dalam penelitian. Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasinya dan apabila ada modifikasi maka harus dituliskan dengan jelas bagian mana dan hal apa yang dimodifikasi.
6. **Hasil**
   Hasil memuat data ataupun informasi utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada suatu tabel/ grafik/diagram atau gambar, maka hasil yang terdapat pada bagian tersebut dapat diuraikan dengan jelas dengan tidak menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata- rata maka harus menyertakan pula standar deviasinya.
7. **Pembahasan**
   Pembahasan bukan merupakan pengulangan dari hasil. Pembahasan mengungkap alasan didapatkannya hasil dan arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, hasil penelitian ini dapat dibandingkan dengan studi terdahulu.
8. **Kesimpulan**
   Kesimpulan berisi infomasi yang menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, dan penelitian berikutnya yang bisa dilakukan.
9. **Ucapan terima kasih**
   Bagian ini berisi ucapan terima kasih kepada suatu instansi jika penelitian ini didanai atau didukungan oleh instansi tersebut, ataupun kepada pihak yang membantu langsung penelitian atau penulisan artikel ini.
10. **Daftar pustaka**
    Pada bagian ini, tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review*. Apabila harus menyitir dari "laporan" atau "komunikasi personal" dituliskan '*unpublished*' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers* dan penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Microsoft Word, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak. Batas kiri -kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan Bahasa Indonesia, angka desimal ditulis dengan menggunakan koma (,) dan ditulis dengan menggunakan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5 cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan international system of units.
4. Nama takson dan kategori taksonomi ditulis dengan merujuk kepada aturan standar yang diakui. Untuk tumbuhan menggunakan *International Code of Botanical Nomenclature* (ICBN), untuk hewan menggunakan International *Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae*, *Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
7. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah. Paragraf pada isi tabel dibuat satu spasi.
8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul gambar ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi, untuk *line drawing* minimal 600dpi.

9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al.* Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al*., 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995). Penulisan daftar pustaka adalah sebagai berikut:
   a. **Jurnal**
      Nama jurnal ditulis lengkap.
      Agusta, A., Maehara, S., Ohashi, K., Simanjuntak, P. and Shibuya, H., 2005. Stereoselective oxidation at C-4 of flavans by the endophytic fungus *Diaporthe* sp. isolated from a tea plant. *Chemical and Pharmaceutical Bulletin*, *53*(12), pp.1565-1569.
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      Merna, T. and Al-Thani, F.F., 2008. *Corporate Risk Management*. 2nd ed. John Welly and Sons Ltd. England.
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      Fidiana, F., Triyuwono, I. and Riduwan, A., 2012. Zakah Perspectives as a Symbol of Individual and Social Piety: Developing Review of the Meadian Symbolic Interactionism. *Global Conference on Business and Finance Proceedings. The Institute of Business and Finance Research, 7*(1), pp. 721 - 742
   d. **Makalah sebagai bagian dari buku**
      Barth, M.E., 2004. Fair Values and Financial Statement Volatility. In: Borio, C., Hunter, W.C., Kaufman, G.G., and Tsatsaronis, K.(eds.) *The Market Dicipline Across Countries and Industries*. MIT Press. Cambridge.
   e. **Thesis, skripsi dan disertasi**
      Williams, J.W., 2002. Playing the Corporate Shell Game: The Forensic Accounting and Investigation Industry, Law, and the Management of Organizational Appearance. *Dissertation*. Graduate Programme in Sociology. York University. Toronto. Ontario.
   f. **Artikel online.**
      Artikel yang diunduh secara online ditulis dengan mengikuti format yang berlaku untuk jurnal, buku ataupun thesis dengan dilengkapi alamat situs dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review misalnya laporan perjalanan maupun artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
      Himman, L.M., 2002. A Moral Change: Business Ethics After Enron. San Diego University Publication. *http:ethics.sandiego.edu/LMH/oped/Enron/index.asp*. (accessed 27 Januari 2008) bila naskah ditulis dalam bahasa inggris atau (diakses 27 Januari 2008) bila naskah ditulis dalam bahasa indonesia

Formulir persetujuan hak alih terbit dan keaslian naskah
Setiap penulis yang mengajukan naskahnya ke redaksi Berita Biologi akan diminta untuk menandatangani lembar persetujuan yang berisi hak alih terbit naskah termasuk hak untuk memperbanyak artikel dalam berbagai bentuk kepada penerbit Berita Biologi. Sedangkan penulis tetap berhak untuk menyebarkan edisi cetak dan elektronik untuk kepentingan penelitian dan pendidikan. Formulir itu juga berisi pernyataan keaslian naskah yang menyebutkan bahwa naskah adalah hasil penelitian asli, belum pernah dan tidak sedang diterbitkan di tempat lain.

Penelitian yang melibatkan hewan
Setiap naskah yang penelitiannya melibatkan hewan (terutama mamalia) sebagai obyek percobaan / penelitian, wajib menyertakan *'ethical clearance approval'* terkait animal *welfare* yang dikeluarkan oleh badan atau pihak berwenang.

Lembar ilustrasi sampul
Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah yang dipublikasi pada edisi tersebut. Oleh karena itu, setiap naskah yang ada ilustrasinya diharapkan dapat mengirimkan ilustrasi atau foto dengan kualitas gambar yang baik dengan disertai keterangan singkat ilustrasi atau foto dan nama pembuat ilustrasi atau pembuat foto.

Proofs
Naskah *proofs* akan dikirim ke penulis dan penulis diwajibkan untuk membaca dan memeriksa kembali isi naskah dengan teliti. Naskah proofs harus dikirim kembali ke redaksi dalam waktu tiga hari kerja.

Naskah cetak
Setiap penulis yang naskahnya diterbitkan akan diberikan 1 eksemplar majalah Berita Biologi dan *reprint*. Majalah tersebut akan dikirimkan kepada *corresponding author*

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MAKALAH HASIL RISET (ORIGINAL PAPERS)

KOMUNIKASI PENDEK (*SHORT COMMUNICATION*)