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PRELIMINARY IDENTIFICATION OF POTENTIAL INDIGENOUS YEASTS FROM NAPA CABBAGE (*Brassica rapa subsp.pekinensis*) WASTES FOR CELLULASE ENZYME PRODUCTION

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Abstract

The aims of the research was to determine the occurrence of indigenous yeasts isolated from napa cabbage (Brassica rapa subsp. pekinensis) wastes potentially in produced cellulase enzyme. Indigenous yeast isolated from 1 g napa cabbage wastes with using modified Potato Dextrose Agar / PDA (Oxoid Ltd.) with the addition of 3% Yeasts Extract / YE (Kraft Foods) and 10 ppm amoxicillin. The identification of yeasts performed under microscope for size and shape then tested with Rapid Yeasts Plus System. Cellulase enzyme production was done with the addition of 2% indigenous yeasts into the substrates in the ratio of napa cabbage and water 1:15 with basal medium contains 0.05% KH_2PO_4 and 0.025% Mg_2SO_4 then incubated for 96 hours at 37ºC (modification of Gupta, et al., 2012). Every 24 hours, 1.5 ml of fermented substrates were centrifuged and the supernatant was taken as crude enzyme. The cellulose enzyme activity determined by measuring the glucose resulted using the DNS method, then tested spectroscopically using UV-Vis 9200 spectrophotometer at 540 nm. Results showed that there are 3 indigenous yeasts isolates that 2 of the isolates identified as Candida krusei and one isolate identified as Hanseniaspora guilliermondii. The cellulase enzyme activity increased in the first 24 hours then decreases until 96 hours. The best isolates activity shown by C.krusei A that resulting glucose contents of 15.9 ppm with the enzyme activity of 0.007U/ml.

Key words**:** *Candida krusei, cellulase enzyme Activity, H. guilliermondi, napa cabbage wastes*

INTRODUCTION

The damaged parts of napa cabbage will throw away and become a waste. Napa cabbage waste contains the same components with napa cabbage that can be utilized. Components contained in the napa cabbage is polysaccharides such as cellulose. Cellulose is known as a structural component of the cell wall composition that is commonly found in napa cabbage. The previous solution in the utilization of waste of cabbage is for animal feed, but there is a more economical and useful alternative that is cellulase enzyme production. Cellulase enzyme can be produced from utilization of cellulose content in napa cabbage waste by microorganisms such as the yeast [8]. The yeast that has the ability to convert cellulose into cellulase enzyme called cellulolytic yeast, either indigenous or non-indigenous.

Cellulase enzymes have the ability to break down cellulose and bioconvert other agricultural wastes. The high content of organic cellulose causes the decomposition process to be longer [5]. The problem can be solved with the use of enzymes so that cellulose decomposition occurs more quickly. Therefore, the cellulase enzyme produced with this napa cabbage waste can solved the problem of unavailability of cheap and efficient enzymes.

MATERIALS AND METHODS

Isolation and Identification of Indigenous Yeasts

Indigenous yeasts were isolated from 1 g of inoculated napa cabbage waste using modified Potato Dextrose Agar (PDA) with the addition of 3% Yeasts Extract / YE (Kraft Foods) and 10 ppm amoxicillin, then incubated for 3 days at 37ºC. Isolates that grow on modified PDAs then identified under microscope for size and shape then tested with Rapid Yeasts Plus System

Cellulase enzyme production

Two percent of indigenous yeast inoculated then incubated with the substrate (napa cabbage) in basal media containing 0.5% KH_2PO_4 and 0.025% Mg_2SO_4 then incubated for 96 hours at 37ºC (modification of [2] and [3]. Every 24 hours, 1.5 ml of fermented substrates were centrifuged at 10000 rpm for 10 min at 4ºC, and the supernatant was taken as crude enzyme.

Enzyme Assay

The enzyme activity was determined by incubating 0.5 mL of supernatant with 0.5 mL of 1% Carboxymethyl cellulose (CMC) solution as a substrate into phosphate buffer (pH 7) for 30 min at 50ºC (Modification of [7]). The reactions was stopped by adding 3 mL of 3,5-dinitrosalicylic acid (DNS) reagent followed by boiling at 90ºC for 10 minutes. The developed color was read at 540 nm using UV-Vis 9200 spectrophotometer. One unit of enzyme activity releases 1µmol reducing sugars (measured as glucose) per mL per min [2].

RESULTS AND DISCUSSIONS

Isolation and identification of indigenous yeasts

Characteristics of colonies grown on the media are shown in Table 1.

Table 1. The Results of Isolate Characterization

Strain	Characteristics
S1.2	Isolate 2 : Round, Smooth, Broken White Colored, Wet, Aerobic
S2.2	Isolate 2 : Round, Broken White Colored, Aerobic
S2.4	Isolate 4 : Oval, Yellow, Anaerobic

Source: Own results.

After characterizing, the cells observed under a microscope to remove other isolates from the yeast. 1 isolates were selected from several isolates having the same characteristics. The results show that the isolates S1.2, S2.2 and S2.4 have similar characteristics, ie tend to be rounded, broken white to yellow and aerobic. Then it was purified until fifth purification because because the colonies grow well. The selected isolates were identified by RapID Yeasts Plus System (Table 2).

Table 2. The Results of RapID Yeasts Plus System with ERIC Analysis

Isolate	S1.2	S2.2	S2.4
Glucose	+	+	+
Maltose	-	-	-
Sucrose	-	-	-
Trehalose	-	-	-
Raffinose	-	-	-
Lipid	-	-	-
NAGA	-	-	-
αGlucoside	-	-	-
βGlucoside	+	-	-
ONPG	-	-	-
αGalactoside	-	-	-
βFucoside	+	-	-
PHS	-	-	-
PCHO	-	-	-
Urea	-	-	-
Prolyne	-	-	-
Histidine	+	+	+
Leucyl-Glycine	-	-	-
Yeast Name	***H.guillermondii***	***Candida krusei***	***Candida krusei***

Source: Own results.

The results showed that 1 isolate was identified as *H.guillermondii* (S1.2) and 2 isolates *C.krusei* (S2.2 and S2.4). *Hanseniaspora guillermondii* is the yeast of the *Saccharomyces* family where the strains of this species produce acetoin or chemicals found in many food products. *Candida* has characteristic cells with size between (2-5) x (2.5-10) µm and varied shapes of round, short oval, oval, oval lengthwise, cylindrical to elongate, rarely shaped apikulat, ogival, triangular or bottle-shaped [4]. Both strains of *Candida krusei* grow with different characteristics and suspected have a different growths.

Cellulolytic Potential of Indigenous Yeasts from Napa Cabbage

The cellulolytic activity determined as a total reducing sugars production. The indigenous yeasts isolates have the ability to degrading the cellulose. [7] Previously reported *Candida tropicalis* has a high activity to degrading a cellulose. Our study noted that *Candida krusei* and *Hanseniaspora guilliermondii*is a cellulolytic yeasts. It seen from the amount of reducing sugar formed that changes every 24 hours, it means that indigenous yeasts

degrades cellulose and produces cellulase enzyme. [10] Reported that reducing sugar formed from cellulose that hydrolyzed by the cellulase enzyme produced by yeast.

The substrate or CMC is converted by crude enzyme into reducing sugar. The reducing sugar produced from enzymatic hydrolysis of cellulose is suspected as glucose because the cellulose metabolism pathway. The mechanism of hydrolysis of cellulose in CMC into reducing sugars occurs via multi-enzyme action [6]. The cellulose metabolism pathway into reducing sugar is the breakdown of cellulose in the form of crystals into cellulose by the endo-cellulase, then the breakdown of cellulose into cellobiose or cellotetrose by exo-cellulase, and cellobiose or cellotetrose then breakdown into glucose by β-glucosidase or also called cellobiase [9].

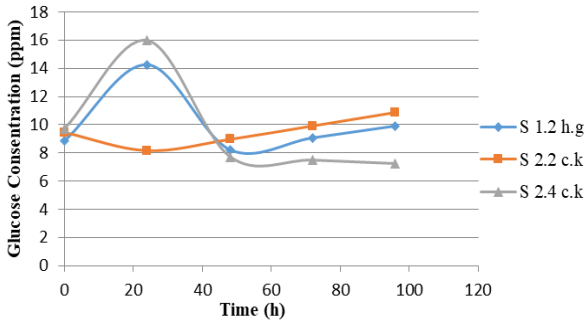

Fig. 1. Glucose Production from CMC by Cellulase Enzyme
Source: Own results.

The highest glucose produced from the substrate by *C.krusei* A (S 2.4 c.k) for 24 hours is 15.9 ppm (Fig. 1). The lowest glucose produced by *C.krusei* B (S 2.2 c.k) at 96 hours with a total of 7.2 ppm. The glucose production graph of *C.krusei* B (S 2.2 c.k) is different from other isolates presumably because the yeast has not yet reached a rapid growth phase. While, graphs of *C.krusei* A (S 2.4 c.k) and *H.guillermondii* (S 1.2 h.g) showed the best glucose production occurred at 24 hours. Each chart is different because each type of yeasts has different optimum growth characteristics.

Determination of cellulase enzyme activity

The results show that for 96 hours, indigenous yeasts can synthesize enzymes or crude enzyme as a supernatant of a centrifuged fermented solution [1]. It shows that the enzyme activity of *H.guillermondii* (S 1.2 h.g) and *C. krusei* A (S 2.4 c.k) increased for 24 hours and then decreased until 96 hours. But the enzyme activity of *C. krusei* B (S 2.2 c.k) decreased until 24 hours and then increased (Fig. 2). *C.krusei* A and *C.krusei* B are two different strains so that their growth (in enzyme production) is also different, where *C.krusei* A can produce many enzymes up to 24 hours, whereas *C.krusei* B can not produce as many enzymes. The production of the enzyme itself is influenced by various factors such as strains and growth rate of strains, available nutrients, temperature, pH, activity water and oxygen availability.

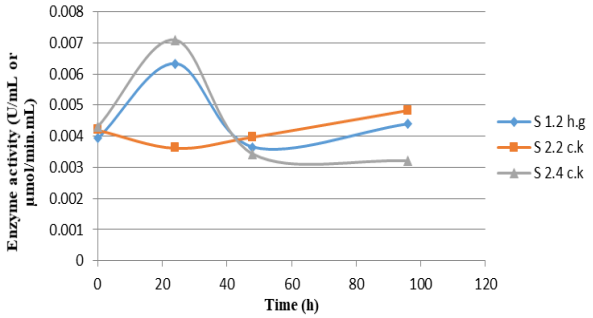

Fig. 2. Cellulase Enzyme Activity from Substrate-Medium Ratio of 1 : 15
Source: Own results.

The highest cellulase enzyme activity was produced by *C.krusei* A (S 2.4 c.k) ie 0.007 U/mL at 24 hours. However, the second *C.krusei* B (S 2.2 c.k) has cellulase enzyme activity below 0.004 U/mL at 24 hours. Then, the cellulase enzyme activity produced by *H.guillermondii* isolate is 0.0063 U/mL at 24 hours. And the smallest cellulase enzyme was produced by C.krusei A ie 0.003 U/mL at 96 hours. The value of cellulase enzyme activity is small because the ratio of substrate-medium is 1:15.

CONCLUSIONS

The results showed that 2 isolates identified as *Candida krusei* and 1 isolate were identified as *Hanseniaspora guilliermondii*. The yeasts are cellulolytic yeasts that have the ability to produce cellulase enzymes. The highest cellulase enzyme activity produced by *C.krusei* A is 0.007 U/mL with a highest glucose production of 15.9 ppm at 24 hours. Then, the cellulase enzyme activity produced

by *C.krusei* B is 0.004 U/mL and by H.*guilliermondii* is 0.006 U/mL.

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