



Screening Cellulolytic Bacteria from the Digestive Tract Snail (*Achatina fulica*) and Test the Ability of Cellulase Activity

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Abstract

On the research of enzyme production levels observed cellulase produced by bacteria in the digestive tract of the isolation of the Snail (*Achatina fulica*). Isolation of bacteria based on the ability of bacteria to grow on CMC media. The purpose of this study was to determine cellulase activity by cellulolytic bacteria. Some bacterial isolates were identified as cellulolytic bacteria, they were KE-B1, KE-B2, KE-B3, KE-B4, KE-B5, and KE-B6. Isolates KE-B6 was the best isolates. Furthermore KE-B6 isolates were grown on media production to determine the pattern of growth and enzyme activity. Measurement of cell growth was conducted by inoculating starter aged 22 hours at CMC production of liquid medium. Cellulase enzyme activity measurements was performed by the DNS method. The results showed that the highest activity by new isolate bacteria KE-B6 and its value of the activity of 0.4539 U/mL, growth rate (μ) 0.377/hour and generation time (g) 1.84 hour. This research expected cellulase of producing bacteria were easy, inexpensive and efficient. This enzyme can be used as an enzyme biolytic once expected to replace expensive commercial enzyme. The biolytic enzyme can be applied to strains improvement (protoplast fusion).

How to Cite

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INTRODUCTION

One of agricultural pests is Snail (*Achatina fulica*) or golden snail. Snail can be found easily in the wild and it has become a major pest of rice which is very detrimental to farmers. Rampant gold snails made farmers look for ways to steer it, the way it's done, among others mechanically by trapping eggs and biologically with direct levy eggs and the golden snail. Musman (2012) states that the golden snail a family with local conch that families Ampullariidae and pink eggs are laid on the surface of the water. Snail (*Achatina fulica*) or Golden snail (*Pomacea canaliculata*) population can be controlled by presenting a predator, that is *Solenopsis geminata*.

Control of snail is supposed not only to get rid of it, but also processes it into something that produces benefits. Research has been done to determine the potential of this golden snail. Bacteria in the digestive tract of golden snail are also reported to have a cellulolytic activity. These bacteria can produce cellulase enzymes to degrade cellulose to glucose. As time, increasing the need of fuel began to be addressed by producing bioethanol, which is one of the renewable fuel source and can be made in a way that is relatively quick when compared with petroleum fuels from non-renewable. Bioethanol has great prospects for this life began to develop ways of production of materials variety.

According to Castro et al. (2002) that the midgut gland of Mollusks Class (Keong Emas), snail capable of producing cellulase enzymes in which there exo and endo glucanase glucanase. While microbes are often found is Bacillus, Brevibacillus, Paenibacillus, Agrobacterium, Pseudomonas and Zymomonas (Wenzel et al., 2002). Ezeronye and Okerentugba (2001) that the intestinal tract of snails normally produce the enzyme biocatalyst glukoronidase β , and β glucanase and endo arylsulphatase.

The presence of bacteria and protozoa in the digestive tract in symbiosis with each other to break down cellulosic or glucan materials (Al-Arif et al., 2012). Based on their ability to digest forages his way and leaf litter, suspected snail (*Achatina fulica*) has the ability to produce the biocatalyst cellulose and glucanase, especially in the digestive tract.

Cellulase enzyme is trivial names with names of systemic β -1,4-glucan-4-glukanohidrolase (EC. 3.2.1.4). Cellulase is a generic name for all enzymes that can break the bonds of β -1,4-glucoside in cellulose, selodextrin, selobisa and other cellulose derivatives (Dini &

Ifah, 2014; Jurick et al., 2012; Sahin et al., 2014). Excluding single cellulase enzyme but an enzyme system consisting of several components of enzymes that work gradually to decompose cellulose into glucose with a composition that varies depending on the source (Jurick et al., 2012)

Cellulolytic activity can be quantified by a variety of methods that have been summarized in recent papers. Since crystalline cellulose is degraded at very slow rates, most assays are adapted to use more easily degradable soluble cellulose derivatives like carboxymethylcellulose (CMC). Screening for extracellular cellulase production by bacteria and fungi is often done on agar plates containing CMC as substrate (Johnsen & Kirsten, 2014). Cellulase enzyme is an enzyme that consists of three main enzyme endoglucanase (CMCase), exoglucanase (aviselase) and glucosidase or selubiase. Cellulase enzymes and glucanase classified as a biocatalyst, the enzyme produced from living organisms, particularly microbes are bacteria, yeast (yeast) and fungi (mold). Selection of cellulase and or glucanase derived from bacteria based on the capabilities and speed the growth of bacteria in terms of producing the enzyme glucanase. One alternative source of biocatalyst can be obtained from the digestive tract bacteria snail (*Achatina fulica*). Biocatalyst (enzyme) cellulase or glucanase of bacteria can be applied to the process of solving the microbial cell wall (protoplast fusion). At this stage, protoplasts obtained in a healthy and normal (Ezeronye & Okerentugba, 2001; Verma et al., 2004; Balasubramanian et al., 2008).

The foremost aim of this study was to found celulolytic bacteria in the digestive tract Snail (*Achatina fulica*) and quantify cellulase activities from them, prompting us to search for a rapid and inexpensive alternative enzyme that fulfilled our need for a suitable assay for fusion of protoplast. Protoplast fusion is one for the strain improvement (Krishnamoorthy et al., 2010)

METHODS

Snail shell initially solved first, then stretched and taken digestive tract part consisting of the mouth, esophagus, stomach down to the anus by using a cutter. The digestive tract that has been taken is then inserted into the mortar that has been sterilized and pulverized using a pestle until smooth, then weighed in the scale.

This bacteria isolate KE-B6 was a collection of the Laboratory of Microbiology. The bacteria was used in the study a bacteria in the intestinal or digestive snail (*Achatina fulica*). The

activity carried out in the Laboratory of Microbiology, Department of Biology, Faculty of Science and Mathematics, University of Diponegoro, Semarang from May to June 2016.

Measurement of cell growth was conducted by inoculating starter aged 22 hours at CMC production of liquid medium aseptically 45 ml. Incubation was performed for 30 hours using a rotary shaker at 120 rpm. Samples were taken at intervals of 0 hours (T0), 4 hours (T4), 8 hours (T8), 12 hours (T12) and 16 hours (T16) by taking 5 ml sample for the measurement of cell growth by measured its absorbance using a spectrophotometer at λ 520 nm and made the curve growth. Culture production medium is then taken 1.5 ml for centrifuged at 3000 rpm for 10 minutes. At this stage it was also determined specific growth rate and generation time (Waites et al., 2001, Dinoto et al., 2006 ; Dinoto et al., 2013).

Media consists of the production of the enzyme (gram / Liter): (0.5) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; (5) $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$; (2.3) NaCl; (20) yeast extract; (10) CMC and distilled water. Bacterial isolates elected KE-B was grown in the medium and was observed every four hours for 16 hours of incubation. Substrate CMC 1% mixed into 0.1 M phosphate buffer, then 3 pieces of test tubes was prepared for each sample with label AS (Sample), AB (Blanko) and AK (Control). Sample tube is inserted CMC substrates of 0.9 ml and 0.1 ml crude enzyme, the control tube is inserted 0.9 ml and 0.1 CMC substrates and crude enzyme reaction stopped immediately into the bath, while the blank tube is inserted 0.9 CMC substrates ml and 0.1 ml of distilled water. The third tube are then incubated at 50 C for 30 minutes, then the reaction is stopped and added back in 1 ml of DNS reagent reheated for 2 minutes. Then added 4 ml of distilled water and its absorbance was measured using a spectrophotometer at λ 570 nm. Cellulase activity can be calculated. The enzyme activity was determined by measuring the amount of enzymes capable remodel 1 micromoles of substrate per minute under certain conditions (OD 570), while the growth of bacteria was carried turbidimetry / OD (520). (Al-Arif et al., 2012; Jurick et al., 2012; Sahin et al., 2013; Johnsen & Kirsten, 2014)

RESULTS AND DISCUSSION

Snails (*Achatina fulica*) is one of the greatest Mollusks and widespread in Indonesia with a body length of about 7-8 cm (Figure 1). Mollusks have a body shell that is strong and rather loud, shaped cone, has a body of flesh, shell rotation

direction clockwise. Body color varies widely, usually depending on the diet, but the dominant color is brown. Terrestrial gastropods like snail, feed primarily on vascular plants. This snail also participates, with other soils invertebrates, in the decomposition of leaf litter (Pawar et al., 2015).



A



B

Figure 1. *Achatina fulica* (A); Part of digestive/intestinum of *A. fulica* (B)

Part gut / digestive snail further blended and reconstituted in sterile distilled water to make serial dilutions. This was done to get the indigenous bacteria in the digestive tract of snails. From the research it, has found some bacteria as listed in Table 1.

Based on the above data, the bacterial isolates KE-B6 selected for the production of cellulase enzymes. This is because these isolates were able to grow in a liquid medium CMC (Media selective) and produce cellulase enzymes highest compared to other isolates. Bacteria or other microbes found in snails, slugs can produce cellulase, glucanase (Castro et al., 2002, Al Arif et al., 2012). Kinds of bacteria obtained from the digestive tract snail as presented in Figure 2.

Table 1. Kind of isolat in the digestive tract of snails

Isolate code	Feature	Enzyme activity (IU)
KE-B1	Ireguler, round, white, shiny, diameter 0.1 mm	0.17
KE-B2	Round, white, shiny white, diameter 0.09 mm	0.23
KE-B3	Round, white, tranparent, shiny, diameter 0.12 mm	0.15
KE-B4	Ireguler, milky, shiny, diameter 0.11 mm	0.24
KE-B5	Ireguler, white, somewhat dingy, diameter 0.12 mm	0.29
KE-B6	Ireguler, round, red, shiny, diameter 0.15 mm	0.39

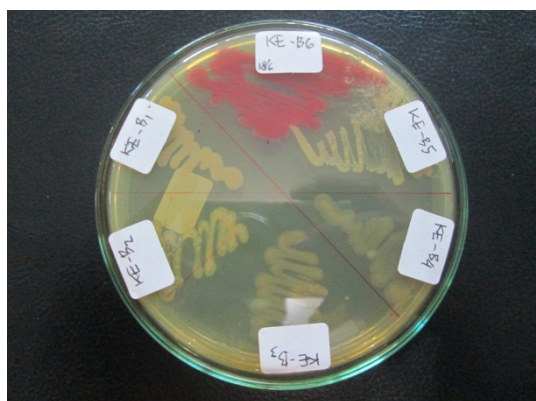


Figure 2. Kinds of bacteria from the digestive tract snail (*A. fulica*)

The growth of isolate bacteria KE-B6

The snail are one of animal mollusks. The snail can use plants as food supplies. In addition, this can use the algae, aquatic plants or agricultural crops. This shows that the snail has the ability to produce digestive enzymes fibers. These enzymes are known as cellulases. In this study isolates KE-B6 is the most potent isolates to produce cellulase. Isolate KE-B6 is a bacteria. It's found in the digestive tract of snails (Figure 3).

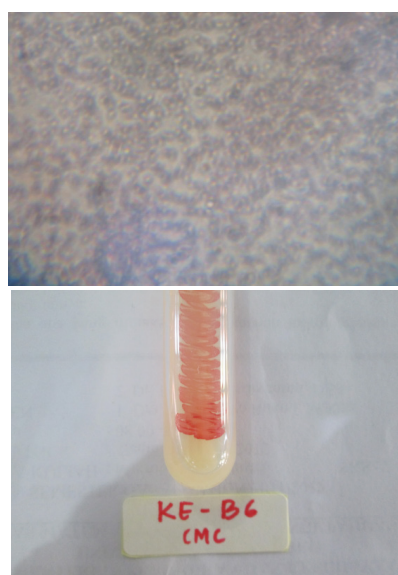


Figure 3. Isolate bacteria KE-B6

Measurements isolate bacteria KE-B6 growth in liquid media carried CMC is used to determine the best time starter pouring into media production. Pouring starter should be done at the time of logarithmic growth. Dwijoseputro (2010) states that the logarithmic phase bacterial cultures took place so quickly that the bacteria that are in this phase is excellent to be used as inoculum. Medium turbidity indicates bacterial growth showed by the absorbance values. Absorbance values obtained by measuring λ 520 nm using a spectrophotometer.

The results showed that the optimal growth occurs in 0-8 hours incubation time belonging to the logarithmic phase. This indicates that the stationary phase began in the incubation time of 8-16 hours. At the time of incubation occurred highest cell growth which generates the absorbance value of 1.39. In Here is a growth curve isolate bacteria KE-B6 based on the time of incubation (Figure 4)

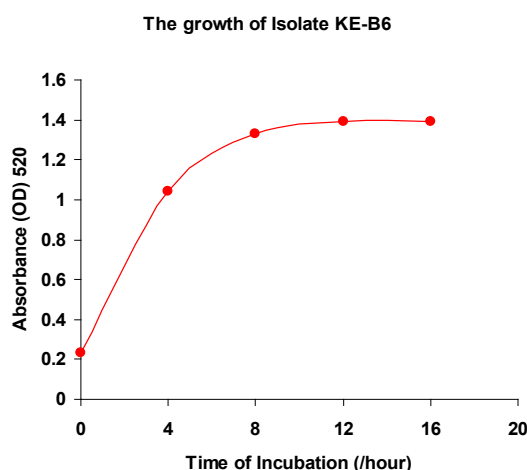


Figure 4. The growth curve of isolates bacteria KE-B6

The growth curve above showed that the treatment time of 0-8 hours incubation showed an increase in the growth and classified into the logarithmic phase (log phase). This phase is formed because the bacteria undergo rapid cell

division and going on cellulose hydrolysis process in the medium. According to Sadhu et al. (2014), cellulase production of *Bacillus* sp best at age 8 hours (incubation period of 2-10 days). But, according to Yang et al. (2014) examination of its growth characteristics *Bacillus subtilis* BY-2 showed that its growth curve entered the logarithmic phase after 8–12 h and the stable growth phase being between 20 and 40 h. This difference is caused by different types of bacteria used, as well as environmental conditions. In this phase of cellulase enzyme formed and are classified as primary metabolites (Madigan et al., 2009; Sadhu et al., 2014). In this phase, cell growth will happen very quickly and the production of enzymes increased (Johnsen and Kirsten, 2014). Based on the log phase of growth patterns, it can be seen specific growth rate (μ) of isolate bacteria KE-B6 that is equal to 0.377 / hr and a generation (g) of 1.84 hours. This value is very important to predict the amount of biomass and the product to be produced (Waites et al., 2001; Madigan, 2009; Dinoto et al., 2006; Dinoto et al., 2013).

Phase lag or adjustment phase of the cell with the environment (medium) does not occur in the growth of isolate bacteria KE-B6, is due to the adjustment phase have been occurred when the starter in the fermentation for 22 hours on a rotary shaker before being mixed in media production. Stationary phase occurs in the incubation time of 8-16 hours, where the absorbance values are stable or decreasing until the death phase. Sadhu et al. (2014) stated that in the stationary phase number of cell will be reduced because of depletion nutrients in the medium. Nutrients are compounds that play a role in the process of energy metabolism and physiology. Nutrients can be a carbon source, a nitrogen source and metal ions contained in media production. The source of carbon in this study is the CMC (Carboxymethyl Cellulose). Nitrogen sources from yeast extract, NH_4NO_3 and macro mineral resources from $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Sadhu et al., 2014).

Cellulase activity of isolate bacteria KE-B6

The amount of cellulose produced by a microbe can be determined by enzyme activity assay. Cellulase activity is the amount of enzyme that liberates as much as 1 mol reducing sugar in the form of glucose from cellulose substrate per minute. Cellulase enzymes typically produced by microbes for example, fungi, bacteria and protozoa but it is also produced by plants and animals (Morana et al., 2011). Enzyme activity test was conducted to determine the amount of cellulose that can be enzymatically hydrolyzed to glucose.

The results showed that treatment incubation time generate the highest activity at an incubation time of 4 hours at 0.4539 U / mL, whereas the incubation time of 0 hours in activity of 0.0655 U / mL, incubation of 8 hours in activity of 0.1002 U / mL, and 12-hour incubation in activity of 0.2319 U / mL. From the research that has been carried out the highest cellulase activity (0.4539 IU) in log phase and classed as inducible enzymes. Inducible enzyme is an enzyme that is created when there is a substrate that acts as an inducer and are extracellular (Bonciu et al., 2011; Johnsen & Kirsten, 2014).

The enzyme activity is generally defined as an amount that may cause a change or transformation as much as 1 mol substrate per minute at a temperature and environment. The enzyme activity was expressed that a single unit of activity (1 U) together with the substrate changes 1U mol / min. The purer will make the higher enzyme activity of a specific enzyme (Okonkwo, 2014). The highest cellulase activity on CMC substrate obtained in the treatment of incubation time 4 hours at 0.4539 U / mL. Dini (2014) states that CMC substrate is a cellulosic substrate amorphous pure form and thus the activity of cellulase enzyme on a substrate CMC enzyme activity is endo-1,4- β -glucanase. This enzyme works on the chain in the CMC to produce oligosaccharides or shorter cellulose chains. The research has been done by Sadhu et al. (2014), that the best carbon source to produce cellulases are substrates CMC, with a pH of 7.0, the incubation time of 8 days and 50 ° C. Graph of the activity of cellulase enzyme isolate bacteria KE-B6 to the effect of incubation time is shown in Figure 5.

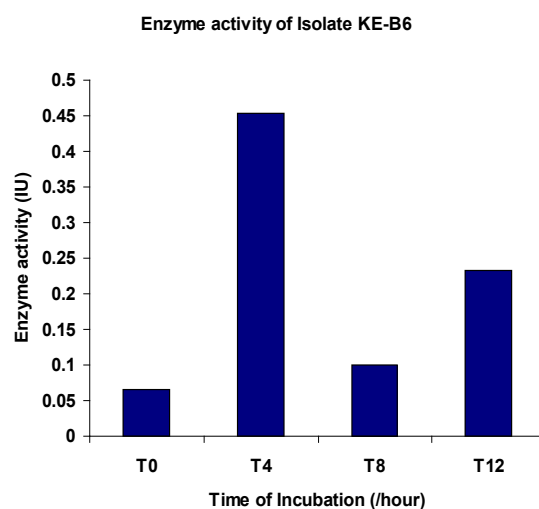


Figure 5. Enzyme activity (IU) of Isolate KE-B6

Cellulase enzyme activity graph by isolate bacteria KE-B6 the effect of incubation time in Figure 4.2. shows that variation of incubation time 0 hours, 4 hours, 8 hours, and 12 hours to produce value activity has increased and decreased or erratic. However, the highest enzyme activity resulting in a 4 hour incubation at 0.4539 U / mL.

Novelity of research is found cellulolytic bacteria in the digestive tract of snails. These bacteria are capable of producing cellulases highest and can be used to break down the cell walls of yeast. This natural enzyme is cheaper and easy to find among other expensive. Another advantage of the natural enzymes is that it can be used as biolity enzyme for strain improvement (protoplast fusion

CONCLUSION

Based on the results of this study, the highest enzyme activity produced by isolate bacteria KE-B6 indicated in the treatment of incubation time 4 hours (T4) with cellulase enzyme activity of 0.4539 U / mL, growth rate (μ) 0.377/ hour and generation time (g) 1.84 hour. Observations using the microscope at 1000 x magnification showed spherical morphology of the bacteria to form short chains and red, so it belongs to the gram-negative bacteria.

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