

# Cellulase induction enzymes characteristic

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## 2 Cellulase induction enzymes characteristics of hindguts of endemic termites of North Sulawesi

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**Abstract.** The necessity for enzyme utilization in various industrial applications and reducing production cost for a more economical process compelled this research to seek for an alternative source of endemic enzyme for various sources cellulolytic enzymes. The termites hindgut is known as a fermentor chamber of its own containing bacterial isolates with the potential to induce cellulosic substrates. Series of research have been conducted to obtain cellulolytic potential bacterial isolates characterized biochemically and molecularly. The purpose of this research was to determine the enzyme characters of cellulolytic bacterial from the hindguts of endemic termites of North Sulawesi. Screening results from various bacterial isolates from hindguts of local termites of several regencies from North Sulawesi identified *Bacillus cereus* from the hindgut of *Odontotermes javanicus* from South Minahasa (North Sulawesi). This *Bacillus cereus* isolate had the best cellulolytic potential by exhibiting a cellulolytic index of 1.75 cm on a selective media of CMC, with the best enzyme production on the 6<sup>th</sup> day with the activity of 0.0054U/ml.

### 1. Introduction

Cellulase enzymes are produced by cellulolytic microbes, namely bacteria and mold. Commercially the price of cellulase enzymes produced from bacterial fungi and fungi is expensive, so the problems that often occur in the enzymatic hydrolysis process are the lack of availability of cheap and efficient cellulose enzymes. The discovery of the new hydrolysis enzyme is an important step to develop the technology of utilizing lignocellulose material to be the starting point for producing biofuel (Lucena et al., 2011). Therefore an attempt is made to find other sources of enzymes that can produce enzyme cellulose.

Cellulase enzymes other than those produced by cellulolytic microbes that live in the wild can also be produced by cellulolytic microbes found in animal bodies. Termites are the most successful group of insects on the planet that can degrade plant lignocellulose biomass. Termites are one of the animals that use cellulose as an energy source, so cellulolytic microbes are found in the digestive tract. Microbial biodiversity in termite intestines can produce enzymes that have the potential to decompose lignocellulose material into biofuel. The use of local microbes in the biofuel production process and adaptation to one another guarantees the successful change in cellulose to ethanol.



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Cellulolytic bacteria are a promising source of new enzymes to produce biofuel based on lignocellulose (Woo et al., 2014; Behera et al., (2014). Therefore, providing renewable source energy is an alternative step that can be taken as an energy source future alternative through efforts to master the processing technology of lignocellulose-based biofuel using cellulolytic enzymes derived from the digestive tract of local North Sulawesi termites.

Many researchers report that cellulose decomposition is carried out by bacteria found in the digestive tract (Breznak and Brune, 1994; Prabowo, et al., 2007; Cho, et al., 2010; Lee, et al., 2010). The ability of cellulolytic activity of several types of termites has been reported by Hetheder, et al., (1991); Purwadaria, et al., (2003); Zhou and Smith (2007) and Lee, et al., (2010), but there is still little research on the characteristics and cellulolytic activity of termite *Odontotermes* sp. The purpose of this study was to obtain cellulolytic enzyme production and characterization of cellulase enzyme activity from the digestive tract of *Odontotermes javanicus* termites based on pH, temperature and induced substrate.

## 2. Methodology

The isolates of *Bacillus cereus* from the digestive tract of *Odontotermes javanicus* termites were rejuvenated on CMC media and grown at room temperature for  $\pm$  48 hours. Making inoculum was carried out by taking 1-2 colonies and growing in 10 ml of CMC liquid media in the test tube, incubated at room temperature for 24 hours. One ml of inoculum was cultivated into each Erlenmeyer flask containing 100 ml of CMC liquid media. Incubation is done at the shaker incubator at a 80 rpm at room temperature. Measurements of enzyme activity were carried out every 12 hours for each isolate. The crude extract enzyme was obtained through culture centrifugation at 2860 rpm for 25 minutes at 40C. The crude extract enzyme is used to test enzyme activity based on pH, temperature and CMC substrate concentration and protein content. Enzyme activity was measured by the formation of reducing sugars using the DNS (Dinitrosalicylic Acid) method (Miller, 1959). Cellulase enzyme activity was obtained based on the  $\mu$ mol of reducing sugar produced per minute. Specific activity of enzymes can be calculated based on the value of enzyme activity obtained divided by the value of protein content (Ghose, 1987).

## 3. Result and Discussion

Cellulolytic enzyme activity produced by cellulolytic bacteria in the digestive tract of *Odontotermes javanicus* termites can be known qualitatively by observing the formation of clear zones formed in 1% CMC induction media which is a common medium used in cellulase activity testing (Lee, 2008). *Bacillus cereus* isolate from Bitung, North Sulawesi has a cellulolytic index of 1.75 cm where the isolates were isolated from the digestive tract of the termite *Odontotermes javanicus*. The formation of clear zones around bacterial colonies showed that cellulolytic bacterial isolates which were isolated from the digestive tract of the tested *Odontotermes javanicus* termites were able to produce cellulase enzymes.

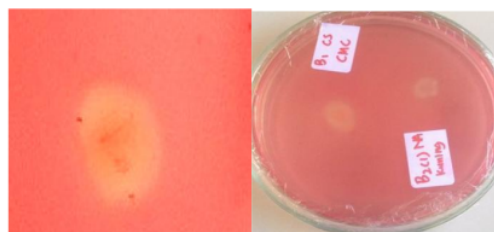
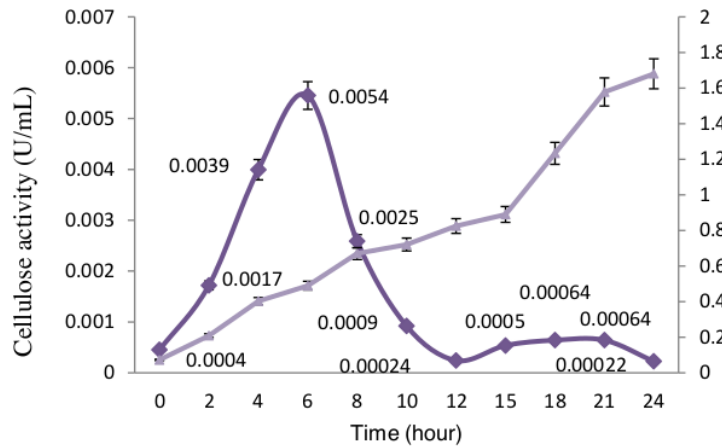


Figure 1. Clear Zone of *Bacillus cereus* in 1% CMC Media

Determination of the optimum time for cellulase enzyme production was carried out by observing the growth of bacterial isolates and cellulase enzyme activity carried out starting from the 0 hour and the second hour then 3 hours to the 24<sup>th</sup> hour. The growth curve of cellulase bacterial isolates and

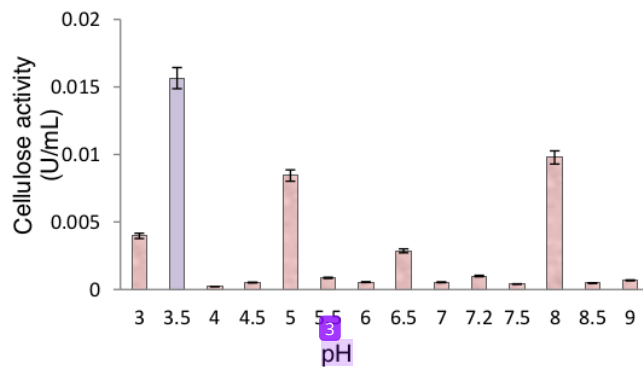
cellulase activity can be seen in Figure 2. From the graph it can be seen that the cellulase enzyme activity shows the peak activity at 6th hour with an activity of 0.0054 U / mL, after the 12<sup>th</sup> hour has decreased with an activity of 0.0024 U / mL, but from the graph it also shows that enzyme activity starts to rise again at the 15<sup>th</sup> hour which is equal to 0.0005 U / mL until the 21st hour with enzyme activity of 0.00064 U / mL. The enzyme activity re-denatured at 24 hours at 0,00022 U / mL so that by looking at the growth pattern, the highest production time of the enzyme occurred at the 6<sup>th</sup> hour.



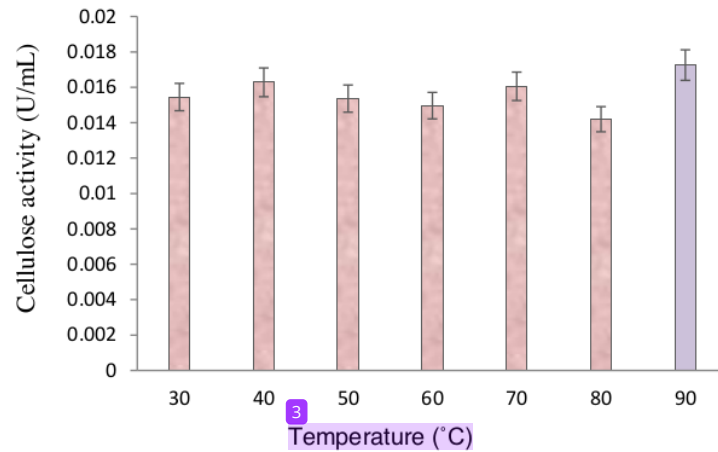
**Figure 2.** Daily Activity of Cellulase Isolate *Bacillus cereus*

**4. Characterization of Cellulase Enzymes**

The cellulase enzyme produced from the cellulolytic bacteria of the digestive tract of *Odontotermes javanicus* termites, was tested in the pH range 3.0-9.0, and the temperature range was 30°C-90°C. The activity of cellulase enzymes reached the highest peak of activity with the Optimum pH of pH 3.5 of 0.015 U / mL. Cellulase activity of *Bacillus cereus* isolates was seen to be quite high at pH 5 of (0.008 U / mL) and pH 8 (0.009 U / mL) (Figure 3).



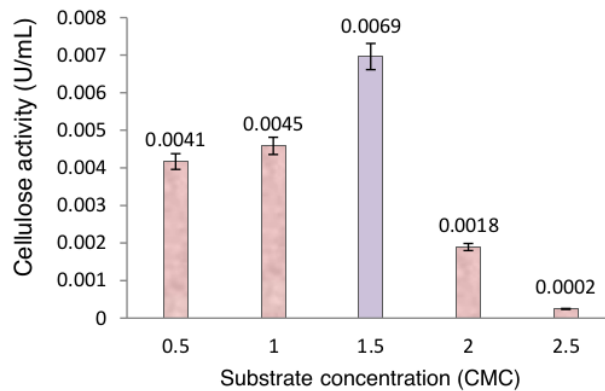
**Figure 3.** Effect of pH on the enzyme activity of *Bacillus cereus* isolates



**Figure 4.** Effect of temperature on the enzyme activity of *Bacillus cereus*

The results of testing the effect of temperature on cellulase activity of *Bacillus cereus* isolates had the highest activity at 90°C at (0.017 U / mL). Cellulase activity *Bacillus cereus* looks up and down at various temperatures.

Cellulase activity of *Bacillus cereus* isolates at various substrate concentrations was carried out using pure cellulose substrate Carboxymethyl cellulose (CMC). The optimum cellulase activity was at substrate concentration of 1.5% CMC with an activity of (0.006 U / mL).



**Figure 5.** Effect of substrate concentration on cellulase enzyme activity isolate *Bacillus cereus*

Cellulase enzymes are enzymes that can hydrolyze cellulose into simple or glucose sugars. Cellulase enzymes are a group of enzymes that can catalyze the hydrolysis of  $\beta$ -1,4-glycosidic bonds in cellulose, selodeskrin, selobiosa, and other cellulose derivatives. The molecule is hydrolyzed into smaller monomer units, such as glucose. This enzyme is able to hydrolyze  $\beta$ -1,4-glycosidic bonds between glycosyl residues through an acidic mechanism (Lynd, et al., 2002). The cellulase structure consists of one catalytic center, cellulose binding region, and glycosylated chain. Cellulase is

classified into three groups, namely endo-1,4- $\beta$ -D-glucanase (EC3.2.1.4), exo-1,4- $\beta$ -D-glucanase (EC 3.2.1.92) and  $\beta$ -D-glucosidase ( EC 3.2.1.21).

Batubara ((1999) reported that the activity of cellulase enzymes and glycosides in the largest termites took place in the back intestine in both *C. curvithus* and *M. gilvus*. This is because there is more symbiosis in the back intestine which contributes to the amount of enzyme extracted. The two symbioses (both bacteria and flagellate) carry out fermentation in the intestine. Meryandini et al., (2009) found four isolates of cellulotic bacteria and measured cellulase enzyme activity.

## 5. Conclusion

Screening results from various bacterial isolates from hindguts of local termites of several regencies from North Sulawesi identified *Bacillus cereus* from the hindgut of *Odontotermes javanicus* from South Minahasa (North Sulawesi). This *Bacillus cereus* isolate had the best cellulolytic potential by exhibiting a cellulolytic index of 1.75 cm on a selective media of CMC, with the best enzyme production on the 6th day with the activity of 0.0054U/ml.

## Acknowledgment

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