Isolation and characterization of Soil Termites (Macrotermes gilvus) cellulolytic bacteria and activity determination of cellulase enzyme on newsprint substrates

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Abstract: The study aims to explore and identify cellulolytic bacteria of termites that have the potential to cellulose hydrolysis on newsprint into glucose. The cellulolytic bacteria are characterized by the formation of clear zones in the medium agar containing newsprint paper after 0.1% Congo red testing. The isolates that produce large clear zones are identified by the type of bacteria. Identification of bacteria morphologically in colonies, gram staining and biochemical tests. The activity of cellulose enzyme based on concentration of reducing sugar (glucose) produced from the cellulose hydrolysis on newsprint was tested by 3.5-dinitrosylicyl reagent using a UV-Vis spectrophotometer. The results showed that 6 cellulolytic bacterial isolates through the Congo red test, 2 isolates that have a large diameter of the clear zone are: Isolates C1 and D1 4.27 cm and 2.05 cm, respectively. Cellulase enzyme crudes of isolates C1 and D1 have an enzyme activity of 15.7 mU/mL and 2.33 mU/mL, protein concentration of 0.35 mg/mL and 1.18 mg/mL with specific activity of 45.17 mU/mg protein and 1.98 mU/mg protein. Based on the observation of the colony’s morphology in macroscopy, microscopy and biochemical test results, an isolate of C1 is the Provedencia sp and D1 isolate is the Bacillus sp.

1. Introduction
Cellulose is the most abundant organic compound on earth. It is estimated that there are around $10^{11}$ tons were synthesized every year. One of the cellulose wastes that has not been used optimally is newsprint waster. Paper waste contains 60-70% cellulose, 10-20% hemicellulose, 5-10% lignin, Newspaper containing lignocellulose which consists of cellulose 40-55%, hemicellulose 25-40%, and lignin 18-30% [1]. Cellulose content is quite high in newsprint, so it has a pretty good potential as raw material for cellulolytic bacterial growth media and functioned as a cellulose substrate that can be hydrolyzed by hydrolase group enzymes to produce glucose. According to References [2] that cellulolytic bacteria are able to live on materials that contain cellulose.

One of the obstacles in lignocellulose hydrolysis is the resistance of lignocellulosic biomass which is very resistant to chemical and biological hydrolysis especially in the crystals of cellulose which compile the nucleus of the plant cell walls [3]. The cellulose conversion into simple sugars requires cellulase enzymes. Cellulase is an extracellular enzyme that is able to hydrolyze cellulose into simple sugar or glucose by breaking the bonds of 1,4-β-glycosidic in cellulose, cellodextrin, cellulbiose, and other cellulose derivatives [4]. Cellulase enzymes consist of endo-1,4-glucanase, echo β-1,4-glucanase, and β-1,4-glucosidase complexes [5]. Generally, cellulase enzymes are only produced by

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microbes both bacteria, protozoa and fungi. The existence of protozoa or symbiotic bacteria in the digestive make it possible for invertebrate and herbivorous animals to digest cellulose [6].

One of the microbes that can produce cellulase enzymes is cellulolytic bacteria. The use of cellulolytic bacteria as a producer of cellulase enzymes in conversion becomes interesting for being studied. These bacteria can break down cellulose into glucose as a source of carbon and energy. The use of bacteria as a enzymes source was chosen because it has several advantages, namely low production costs, can be produced in a short time, has a high growth rate and is easily controlled [7].

Enzymes are produced by microbes that digesting food. One of them is coming from insects. But most of insects are assisted by various microorganisms found in the digestive system. The digestion process of carbohydrates, proteins and lipids occurs in the middle intestine (midgut), although part of the digestion of cellulose caused by microbial cellulase enzymes also occurs in the hind gut [8].

Interesting facts were observed by an observations that were doing by a type of insect that lives on wood and makes it as its food. The results of the initial identification indicate that these insects belong to the type of termites. The ability of these insects to eat wood is interesting to be studied, especially the bacteria that are symbioned in the digestive system.

Termites are one of the most abundant macro invertebrates and play the important role in ecology in tropical ecosystems. Termites are able degrading cellulose because in the digestive tract there are symbiotic microorganisms such as bacteria and protozoa. The existence of symbiotic bacteria in the digestive make it possible for termites to digest cellulose. In the digestive tract of termites *Mastotermes darwiniensis* can be found bacteria *Promicromonospora citrea*, *Promicromonospora sukumoe* and genus *Cellulosimicrobium*, *Clostridium cellulovoran* that able produce complex cellulase enzymes [9].

Insects that digest wood components as nutrients are often helped by microorganisms by taking microbial-generated enzymes on digestible substrates, removing and detoxifying toxic substances in wood, microbes that live in intestines produce and release enzymes, and microorganisms that work as decomposers and release major carbon sources [10].

Cellulolytic microorganisms such as bacteria and fungi produce enzymes that can hydrolyze cellulose into oligosaccharides, at the end become glucose which functions as a source of carbon and nutrients for the growth of these microorganisms. The enzyme that plays a role in the hydrolysis process is cellulose [11]. The use of cellulase enzymes is currently increasing in the activities of the food and beverage industry, paper, detergents, and agricultural industries [12].

Cellulolytic bacteria have been isolated from various sources such as sourced from the soil [13] agricultural fields in India [14], cow feces [15]. Cellulolytic bacteria have also been isolated from insects [16], wood beetles [17] pine and adult beetle larvae [18] butterfly larva *Cossus Cossus* [7].

References [19] who succeeded in exploring cellulolytic bacteria from worker termites and obtained 4 cellulolytic bacterial isolates successfully isolated from the intestines of worker termites *Macrotermes gilvus* using CMC substrates. These isolates have anaerobic characteristics. Bacterial isolate with code RA6 has a cellulolytic activity index of 2.5 U/mL with a clear zone diameter of 5 mm. Based on the results of Gram staining, all the isolates were Gram negative bacteria which were marked with pink, with the form of kokus cells and short stems. From the results of the research conducted it was found that these bacteria included the type of *Paracoccus yeei*.

Cellulolytic bacteria have many benefits, such as renewable energy renewal, in agriculture and animal husbandry. So it is not surprising that many researchers are researching the existence, characterization and even how to use it with various modifications. Thus in this study an exploration of cellulolytic bacteria is coming from soil termites with bacterial growth media by using newsprint substrate.

2. Research Methods

2.1 Isolation of cellulolytic bacteria from soil termites (*Macrotermes gilvus*)

Termites are sterilized by dipped it in 70% alcohol for 30 seconds. The stomach part of termite is cut using a sterile knife, ejected liquid using ose needles, then scraped into medium agar with a composition of 2% bacto-peptone, 1% bacto yeast extract, 1% NaCl, 2% bacto agar, and distilled
water, then incubated for 24 hours at 37 °C. Bacterial colonies which grows and has different morphological characters each taken one ose and then scratched into a medium to be selectively cellulolytic with a composition of 0.02% MgSO₄; 0.075% KNO₃; 0.05% K₂HPO₄; 0.002% CaCl₂; 0.2% yest extract; 1.25% bakto agar, and 1% newsprint powder and aquades, then incubated for 24 hours at 37 °C. Colonies that grow are scratched quadrants until pure isolates are obtained. The pure isolate was taken from one ose and then sprinkled on a medium agar of newspaper, then incubated for 24 hours at 37 °C. Before testing each isolate, the culture stock was prepared on the medium so that it tilted. This needs to be done because the congerad testing makes bacterial isolates died because the congored is disinfectant. The bacterial isolates that grew was dropped of 0.1% congo red solution, if there is a clear zone around the colony, that are indicated as isolates of the cellulase enzyme producing bacteria. Furthermore, pure isolated bacteria that has clear zones is regenerated on the cellulolytic selective medium, to determine the cellulolytic index, a clear zone is measured by using calipers. Bacterial isolates that have large clear zone diameters and have good growth order are subsequently characterized.

2.2 Characterization of cellulase enzymes producing bacterial from Soil Termites (Macrotermes gilvus)

2.2.1 Morphological observations in macroscopy scale
Morphological observations in macroscopy scale by observing colony shape, colony surface is observed from the side, the edge of colony is observed from above, colony color, cell shape, and cell arrangement that grows on the Nutrient Agar medium.

2.2.2 Microscopic observation of morphology
Cell morphology observation was carried out by gram staining technique. Culture of isolates of one ose aged 24 hours was made by smear preparations on glass objects using physiological NaCl. Then the preparation was dried at room temperature then it was fixed above the bunsen. Then the dried preparations was dripped with Gram A solution (crystal violet) and left for 3 minutes, then rinsed with running water. After that, it is dripped with a solution of Gram B (lugol) and left for 1 minute, then washed with running water. Furthermore, it is dripped with Gram C (alcohol) until the remaining dyes are removed and rinsed again with running water. The final stage of the preparation is dripped with Gram D (safranin) solution and left until it dried. Then the dry preparations were dropped by immersion oil for being observed under the microscope with 1000x magnification. Observations were made by looking at cell morphology and color. Gram-positive bacteria are purplish blue, while red gram-negative bacteria are easy [20]

2.2.3 Biochemical tests of selected cellulolytic bacteria
Biochemical tests of selected cellulolytic bacteria isolates were carried out based on the methods in Bergey's Manual and Systematic of Bacteriology in References [21] as follows:

2.2.3.1 Simon Citrat Agar (SCA) test
Simon Citrat Agar test was carried out by taking one ose of selected bacterial isolate, scratched on the surface of Simon Citrat medium Agar and also 1 ose of bacterial isolate injured into the middle of the media to the bottom of the test tube. The culture medium was incubated at 37 °C for 48 hours. Positive test if the medium changes from green to blue (temperature modified).

2.2.3.2 Catalyst test
Hydrogen peroxide 3% was dropped by 2 drops on the glass object, then one ose of bacterial isolate was lied on top of it. Positive catalase test are characterized by the formation of air bubbles. The presence of air bubbles shows the amount of oxygen gas produced, this means that the enzyme is able to decompose H₂O₂ into H₂O and O₂
2.2.3.3 Test of Triple Sugar Iron Agar (TSIA)
The bacterial isolate is scratched on the surface of the medium to tilt TSIA and also pierced perpendicular to the middle of the medium. Then incubated for 48 hours at 37 °C, a positive test indicated a change in the color of the TSIA medium from dark brown to orange or yellow. The formation of H₂S can be observed with the formation of blackish color and scratches, gas formation can be observed with formation a cavity at the bottom of the agar (temperature modified).

2.2.3.4 Motolitas test
Bacterial isolate of one ose was inoculated on the Indol Motility Sulfite (SIM) medium by injected it into the medium until reached half in the test tube, then incubated for 48 hours at 37 °C. Positive tests are indicated by traces of bacterial movements (temperature modified).

2.2.3.5 Methyl Red (MR) and Voges Proskauer (VP) test
The bacterial isolates of one ose were inoculated on MR and VP medium, then incubated for 24 hours. For testing Methyl Red, the inoculum is dropped 2 drops of Methyl red, if a purple-red ring is formed it shows a positive reaction. For the test Voges Proskauer, the inoculum is dripped with 2 drops of barite A and B barite reagent, if a purple-red ring forms it showed a positive reaction.

2.2.3.6 Urea test
Test is done by inoculating 1 ose the culture of bacteria into a medium containing urea and phenol red indicator. A positive test is indicated by the change of color of the medium from red orange to red purple. Directions for urea hydrolysis, identify urease-producing bacteria that can hydrolyze urea. When urea is hydrolyzed, NH₄⁺ accumulates in the medium and causes the pH of the medium to become alkaline.

2.2.3.7 Carbohydrate fermentation test
Carbohydrate fermentation test are carried out by inoculating one bacterial culture into a medium consisting of glucose, sucrose, maltose and lactose. Positive test are characterized by the change of the color of the medium to yellow.

2.2.4 Identification
The characterization results of each bacterial isolate were identified using book of Bergey's Manual of Determinative Bacteriology [21].

2.3 Determining the time of bacterial growth
Growth determination of bacteria aimed to know the optimum production time of enzymes (proteins) and carried out in the fermentation process in a liquid medium containing newsprint paper as a substrate. The inoculum which has been fermented for 24 hours at 37 °C, was taken 10% for being inoculated into a liquid medium containing 2% newsprint paper with a total volume of 100 mL, then is incubated in a shaker incubator with agitation speed of 200 rpm for 40 hours at 37 °C. 8 mL was taken every 8 hours and optical density (OD) and cellulase activity were measured. Optical density (OD) was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm. The growth curve is determined by making a plot between time and optical density [22]. While cellulase activity was determined by measuring reducing sugar levels with the DNS method [23].

2.4 Isolation of cellulase enzymes from bacterial symbiont isolates of Soil Termites (Macrotermes gilvus)
The bacterial isolates that have a clear zone, 24 hours old were taken 2 ose inserted into erlenmeyer containing 100 mL of inoculum medium, then incubated in a shaker incubator at 200 rpm for 16 hours at temperature 37 °C. Then the active inoculum was taken 10% and put into erlenmeyer containing 500 mL of production medium, then it was incubated in an incubator shaker with agitation of 200 rpm for 24 at 37 °C. After that centrifuged at 3500 rpm, 4 °C for 30 minutes to separate the supernatant and
bacterial cells. Supernatant is a rough cellulase enzyme crude, then enzyme activity was tested by the DNS method [23] and determination of protein content by the Lowry method [24].

2.5 Determination of cellulase enzyme activity from bacterial symbiont of Soil Termites (Macrotermes gilvus)

Test of cellulase enzymes in principle is based on the amount of reducing sugars produced from hydrolysis of cellulose. The growth medium uses a liquid medium containing newsprint paper. The produced reducing sugar is calculated as glucose by the DNS method [23] A mixture of 0.5 mL of a rough cellulase enzyme solution, 0.5 mL of buffer of sodium phosphate pH 7 and 0.5 mL of 1% newspaper pulp substrate was incubated at 37 ° C for 60 minutes. After that the mixture is added 1.5 mL DNS reagent, then shaken with vortex for 10 seconds. Then the mixture is heated in boiling water for 10 minutes, then cooled in ice water. The reaction results were measured for absorbance at the maximum wavelength (λ=450 nm). Glucose levels from cellulose hydrolysis by cellulase enzymes can be calculated using a standard glucose solution calibration curve in the range 0-0.16 mg / mL. Calculation of glucose levels is done by substituting the absorbance of the solution which was obtained in determining glucose levels in the linear regression equation. The glucose level obtained is then used to determine enzyme activity using the formula for calculating cellulase enzyme activity in equation (1).

\[
\text{Enzyme Activity (Unit/mL) = \frac{\text{glucose level} \times F_p}{BM \times V \times T} \times 1000 \, \mu\text{mol/mmol}}
\]

Description:
- \(F_p\) : dilution factor
- \(BM\) : glucose molecular weight
- \(V\) : volume of used enzyme (mL)
- \(T\) : incubation time (minutes)

The enzyme activity obtained is expressed in Units / mL, one unit of cellulase activity is a number of enzymes that produce 1 µmol of reducing sugar (glucose) per minute under certain conditions.

2.6 Determination of protein levels

Protein levels are determined based on modified Lowry method [24]. Mix 1 ml of enzyme solution, 5 mL of Lowry B reagent shaken with vortex for 10 seconds. Then the mixture was left at room temperature for 15 minutes, then 1 mL Lowry A was added and shaken with vortex for 10 seconds. After that the mixture was left at room temperature for 30 minutes so that the reaction runs perfectly, then added 3 mL of distilled water. Standard protein solutions use BSA in the range 0-0.16 mg/mL and distilled water as blank. Absorbance is measured using a spectrophotometer at a maximum wavelength of 640 nm.

Calculation of enzyme protein levels is done by substituting the absorbance of the solution which obtained on determination of enzyme proteins level in the regression curve equation. The enzyme protein levels was obtained then used to determine enzyme-specific activities using the specific activity calculation formula in equation (2).

\[
\text{Specific Activity (Unit/mg protein) = \frac{\text{Enzyme Activity}}{\text{protein level}}}
\]

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3. Results And Discussion

3.1 Isolation of bacterial symbiont of cellulase enzyme producing bacteria from the soil termites (Macrotermes gilvus)

The result of bacteria isolation using the scratch method on the surface of the medium to obtain a bacterial colony of varying shapes. Then colonies that have different morphologies are purified by quadrant method. The pure bacterial produced were grown in a cellulolytic selective medium (containing newsprint powder) at 37 °C pH 7 and a 24-hour incubation time, resulting in 8 isolates. The six isolates that grow can hydrolyze cellulose on newsprint substrate at 37 °C pH 7 incubator time 24 hours with different hydrolysis abilities. The cellulase enzyme producing bacteria are characterized by the formation of clear zones in medium containing newsprint substrate after 0.1% of congo red drops. These isolates have different abilities in hydrolyzed cellulose on newsprint as the substrate. Isolates with high cellulolytic activity have large clear zones. The six isolates of the cellulolytic bacteria were selected two of which have a large clear zone that is C1 and D1 isolate with a clear zone of each 4.75 cm and 2.05 cm (Figure 1).

![Figure 1](image-url)  
Figure 1. Two selected isolates based on larger clear zone diameter after 24 hours incubation at 37 °C, C1 isolates and isolate D1

3.2 Characterization of enzyme producing cellulolytic bacterial from soil termites (Macrotermes gilvus)

3.2.1 Macosscopic morphological observation, gram microscopy

Based on the morphological observations of in macroscopy on colonies of isolate C1 and isolate D1 by observing the shape, edges and color of bacterial colonies, both bacterial isolates from termites have a convex round shape with ridged edge colonies, clear white colony . observation of Gram staining of isolate C1 and isolate D1 with microscope with the aim of knowing bacterial cell morphology and its Gram properties. The overall observations are shown in Table 1.

| No | colony morphology | C1 | D1          |
|----|-------------------|----|-------------|
| 1  | Colony color      | Clear | white       |
| 2  | Colonies          | Convex Round | convex spherical |
| 3  | colonies edges    | Jagged | Jagged     |
| 4  | cells form        | Basil | Basil      |
| 5  | Colors Gram       | red | purple     |
| 6  | Gram nature       | Negative | positive  |
| 7  | Surface colonies  | Spread | Spread     |
Based on the data in Table 1 indicate that there are two types of bacteria from gram staining results. C1 isolates are Gram-negative bacteria because the color formed after the addition of crystal violet is red. D1 isolates are Gram-positive bacteria because they are purple after adding violet crystals.

### 3.2.2 Biochemical tests observation

The characterization of most microbes such as bacteria is based on enzymatic reactions and biochemical reactions. Microbes can grow on several types of medium to produce certain metabolites that are detected by microbial extraction with certain reagents that produce color changes in the media. Reactions in microbial cells will be identified by carrying out certain test. Bacteria in nature, especially bacteria that are symbiotic with termites, have different characteristics. The observation of biochemical tests of cellulolytic bacteria isolates and isolate C1 and D1 shown in Table 2.

| Observations | Isolate C1 | Isolate D1 |
|--------------|------------|------------|
| SCA Test     | Positive   | Negative   |
| Catalase Test| Positive   | Positive   |
| TSI test     | Positive   | Positive   |
| Motoloty test| Positive   | Positive   |
| Sulphide test| Negative   | Negative   |
| Indole test  | Negative   | Negative   |
| Gas test     | Negative   | Negative   |
| MR test      | Positive   | Positive   |
| VP test      | Negative   | Negative   |
| Urea test    | Positive   | Positive   |
| Glucose test | Positive   | Positive   |
| Lactose test | Negative   | Negative   |
| Sucrose test | Positive   | Positive   |
| Maltose test | Negative   | Negative   |

Biochemical properties character of bacterial isolates from soil termites in Table 2. The motility test using Sulfide Indol. Motility medium showed that isolates C1 and D1 were motile characterized by traces of bacterial movements, positive SCA test was marked by changes in the color of medium from green to blue, Isolate C1 was able to use citrate as a carbon source. While D1 isolate are not able to use citrate as a carbon source.

Methyl-Red (MR) test on two isolates showed negative results because when 2 drops of methyl red given on the VP medium did not form a purple ring. Same as did the addition of 2 drops of Barite A and B reagents on the medium did not form a reddish purple ring.

Negative gas formation test because it is characterized by did not form a cavity at the bottom of agar. The Voges Proskauer test (VP) test, both isolates showed a positive result because when given 2 drops of methyl red on the MR-VP medium it formed a purple ring, as well as adding 2 drops of Barite A and B reagents to the medium formed a reddish purple ring.

Catalase test results with the addition of 3% of H₂O₂ solution indicated that isolates C1 and D1 have the catalase enzyme because of the formation of air bubbles around the bacterial colonies. The catalase enzyme can decompose H₂O₂ into H₂O and O₂.
Carbohydrate fermentation tests include glucose, sucrose, maltose, and lactose tests. Isolates C1 and D1 in the glucose fermentation test, sucrose showed a positive result because the change of the color of the medium from green to yellow after incubation for 24 hours, while lactose fermentation, medium color maltose remained green showed negative results. Isolates C1 and D1 have the ability to use glucose, sucrose as a carbon source.

Based on the results of analysis in Tables 1 and 2 after being matched with identification keys and guided by *Bergey's Manual of determinative Bacteriology*, cellulolytic bacteria isolate D1 has the similarities and morphological, physiological, and biochemical characteristics with *Bacillus sp.* whereas cellulolytic bacteria isolate C1 has similarities and morphological characteristics, physiology, and biochemical tests with *Providencia sp.*

3.3 Observation of bacterial growth of C1 and D1 isolates

The growth of microorganisms can be defined as an event of increasing an organism's volume with the increasing of biomass cell [7]. The making a bacterial growth curve aims to determine the time of the exponential phase (logarithmic phase) in order to isolate the cellulase enzyme, and find out the effect of fermentation time on bacterial growth (OD) and cellulase production which has maximum cellulase activity. The bacterial growth curve of C1 and D1 isolates is shown in Figure 2 and Figure 3.

![Figure 2](image-url)  
**Figure 2.** Bacterial growth curve isolates C1 and curve cellulase activity in condition: Medium containing 2% newsprint, pH 7, 37 °C, agitation 200 rpm for 40 hours, using 1% newsprint substrate.

Optical Density (OD) and cellulase activity measurements from C1 and D1 are shown in Figure 2 and Figure 3. Based on the data in the figure, the 8th to 16th hours are logarithmic phases, in this phase bacterial cell growth very fast. In this phase, bacteria divide exponentially. Nutrition in the production medium containing newspaper is used by bacteria to grow and divide. In the logarithmic phase, bacterial cells are very active in splitting and the cell metabolism is rapid, the optimal growth of bacterial cells is in this phase. In optimum condition of growth, the cell splitting in an extraordinary amount in a short time. According to references [25], the growth rate during the logarithmic phase is determined by several factors such as incubation temperature, agitation speed, and pH of the planting medium. At the 24th to the 32nd hour is the stationary phase. At the stationary phase, the cell breeding rate is the same as the cell death rate so the total number of bacteria is the same. The speed slows down because nutrient was reduced which contained in the newsprint medium, the maximum number of bacteria in the logarithmic phase so that cellulase enzymes will be maximized.
Figure 3. Growth curve of bacteria isolates D1 and curve cellulase activity in condition: Medium contains 2% of newsprint, pH 7, 37 °C, 200 rpm agitation speed for 40 hours, using 1% of newsprint substrate.

The C1 and D1 isolates have maximum cellulase enzyme activity at 16th hour or at logarithmic phase. Cellulase enzyme was secreted at 8th hour with enzyme activity of 8.365 mU/mL for C1, and for D1 was 1,003 mU/mL, then enzyme activity increased to 16. 39 mU/mL for C1, and D1 produced cellulase enzyme with an activity of 1.64 mU/mL at 16th hour, and then a decrease in cellulase secretion at 24 hours and above. This shows that the optimum time for cellulase production from the two bacterial isolates was at the 16th hour.

The time required for the production of cellulase enzymes from symbiont bacteria of termites for C1 and D1 in contrast to the time required for the production of cellulase from bacterial symbionts butterfly larvae ie 24 hours using the medium of CMC and substrate CMC 1% [7].

3.4 Isolation of cellulase enzyme and determination of specific activity of cellulase enzymes

The isolates of C1 and D1 were analyzed further to produce cellulase enzymes extracellularly. They were inoculated on the production medium containing newsprint paper each fermented for 16 hours, then centrifuged at a speed of 3500 rpm for 30 minutes, the supernatant produced was a rough extract cellulase enzyme. The enzyme was determined by its activity in hydrolyzing cellulose to glucose on newspaper substrate and its protein content was determined. Data on enzyme activity and protein content are shown in Table 3.

| No | Bacterial Isolates | [protein] (mg/mL) | Enzyme Activity (mU/mL) | Specific Activity (mU/mg protein) |
|----|--------------------|------------------|------------------------|---------------------------------|
| 1  | D1                 | 1.1767           | 2.33                   | 1.9801                          |
| 2  | C1                 | 0.3476           | 15.7                   | 45.1668                         |

Based on the data in Table 3 shows that the two isolates can produce cellulase enzymes that are able to hydrolyze cellulose to glucose on newspaper substrate. Cellulose enzymes from C1 has higher enzyme activity and activity than cellulase enzymes from D1. When associated with clear zones produced on solid media containing newsprint, C1 and D1 isolates have clear zone diameters of 4.75 mm with enzyme activity 15.7 mU/mL and 2.05 mm respectively with activity of enzyme is 2.33 mU/mL. So the greater the diameter of the clear zone produced by microbes, especially cellulolytic bacteria, the greater the activity of the enzyme produced. Data from the protein level analysis used to
determine specific activity to its substrate on enzyme. Enzyme is biocatalyst works specifically to its substrate. The used substrate in this study is newsprint which containing 40-50% of cellulase [1]. The specific activity of sellulase enzyme of C1 is equal to 45.17 mU/mg of protein.

4. Conclusion
Based on the study, we can conclude the soil termites potentially produce cellulotic bacteria. There are 6 isolates grew on newsprint selective medium. Bacterial isolates produce sellulase by 0.1% of congro red test. 2 isolates were chosen that were C1 and D1 isolates, which have clear zone diameter on each diameter 4.75 cm and 2.05 cm, with cellulase enzyme activity to newsprint paper that 15.7 mU/mL for C1 and 2.33 mU/mL for D1. 0.35 mg/mL with specific activity of 1.18 mg/mL with specific activity 1.98 mU/mg protein for D1. The result of characterization microscopically by gram staining showed that C1 is include negative Gram bacteria, and D1 is include in positive Gram bacteria. Both of isolates are basil shape, based on morphology observation of the colony in macroscopy, and microscopically and the result of biochemical tests that C1 is include in *Provedencia sp* and D1 is include in *Bacillus sp*.

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