Screening assays of termite gut microbes that potentially as probiotic for human to digest cellulose as new food source

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Abstract. According to UN, earth population will increase approximately 7.3 billion people up to 11.2 billion from 2015 until 2100. On the other side, food needs are not balance with the availability of food on earth. People of the world need solution for a new food source. By cellulose digesting ability, people analyzed can consume cellulose as the new food source to get glucose. The aims of research is obtaining termite gut cellulase bacteria selected which is potential as probiotic to split cellulose. Method used was as follows; isolation of termite gut microbes, microbial cellulase purification by screening method and probiotic test includes microbial pathogenicity test and human stomach acid and salt osmotic concentration resistance test. The result shows, 3 pure isolates of termite gut microbes can break down cellulose in the medium 1% CMC and 0.1% congo red (indicator of cellulose degradation activity) and life at pH 2-2.5 and osmotic salt condition. Two isolates show the activity of gamma hemolysis (non-pathogenic in terms of pathogenicity on human blood). In conclusion, there are isolated termite gut microbes can be used as probiotic candidate for human to digest cellulose of the new food source for global food scarcity era.

1. Introduction
Nowadays, the world’s concern about the population and food needs are increasing over the year. It is based on data reported by FAO/Food and Agriculture Organization that consumption needs of the people in the world, especially sugar, will increase continuously until 2050 with are about 230 million tons [1].

The increasing of population and consumption of staple food needs is not equal with the global food availabilities. Decreasing of global food availabilities is caused by disappearing of the food supply and castaway as a waste. It will cause inefficiency of processing and managing of food materials [1, 2].

Global warming go on annually, and reported to be responsible as factor of global food scarcity in the future [3]. Agricultural land degradation also needs to be concerned for the next agriculture [4]. Global people need a solution. Grasses can live everywhere even in extreme region [5]. As far as, grasses aren’t intended for food content, it’s intended for the production of bioethanol and the alternative of animal feed, especially families of Cyperus. However, grasses are known to have high cellulose (40-55%) [6], that is polimer of carbohydrate consist of glucose monomers binded by β (1→4)-glycosidic bonds [7].

Cellulose cannot be digested by human because there is no cellulase enzymes in human gut digestion. It means, human do not have enzymes like termites [8]. Termites can digest cellulose as it is mutually symbiotic with particular microorganisms which produce specific enzymes [9]. Cellulase changes cellulose materials into fermentable mono- and oligo-saccharides monomer [8]. Recently, despite fuel and textile industry, the uses of cellulase bacterial are still only glued to improve the digestive efficiency
of ruminant animals, [10]. On the other hand, bacteria that is useful for the host like animal or human in digestion is known as probiotic [11]. Probiotics are traditionally related with fermented foods and from bifidobacteria and lactobacilli. However, the development of probiotics has been made recently [11].

The research efforts to make grasses become a new food source in the future needs be done. Using the termite gut microbes selected as cellulose probiotic as a natural splitter of cellulose for human’s body, is the solution where it aims to increase the efficiency of human’s digestive system in the future, in the global food scarcity.

2. Methods

2.1. Sample Collection and Isolation
The termite samples were obtained in the Gondang village of Semarang. Coptotermes sp were taken from decaying wood in the shade side. The preparation of isolation was carried out by preparing selectively modified NB media with 1% of CMC / Carboxymethylcellulase [12]. CMC is used as a substitute of cellulose polymer [13]. Isolation was carried out by washing the termites for 4 times aseptically in Alcohol 70% for 5 minutes, Aquadest and Alcohol 70% for 30 seconds. Termites were separated between the abdomen and the superior part with a sterile knife. The extract was transferred to the NB + CMC medium and incubated for 4 days in the 120 rpm 37°C [12]. Then, bacterial culture was prepared to transferred in medium NA + CMC. The pouring was carried out by graded dilution technique using oxalate salt solvents [14]. Bacterial culture were incubated for 48 hours 37°C.

2.2. Cellulolytic test
Isolated spesific bacteria had tested in the cellulase screening. The medium was made by regulating the acidity level for 2-2.5 (as an acid resistance test in stomach) [15]. Medium enriched with 1% of CMC and 0.1% of Congo red cellulolytic indicator reagent [14]. Isolate was transferred to the test medium using streak technique. The culture was incubated for 48 hours 37°C in the incubator. The grown isolates with a clear zone indicates that the bacterial isolates passed the screening [15].

2.3. Pathogenicity Test
Isolated cellulase bacteria were inoculated into the new blood medium (Blood Agar base + 5% of type O of human blood (sterile) using the streak technique [16]. Human blood and blood agar base were obtained from Laboratory of Diponegoro Hospital. The isolated in the blood medium were incubated for 48 hours and 37°C. Isolates that didn't show the clear zones and didn’t change the color of the blood medium are identified as non-pathogenic bacteria or gamma hemolysis [16]. Probiotics bacteria must be non pathogens, so that were not harmful when consumed [17].

2.4. Human Stomach Acid and Salt Osmotic Concentration Resistance Test
The escaped isolates were inoculated into test medium before incubated for 48 hours by acid treatment on disk paper, pH 7 and pH 2.5. The isolated bacteria was also inoculated into 3.5% and 6.5% (w / v) of sodium chloride solution, then incubated for 2-4 hour 37°C [18] and poured into NA + CMC medium for 24 hours. Starch agar plates containing different sodium chloride concentrations were used to analyze the sodium chloride tolerance of the bacteria [19]. The bacteria resistance were showed by the grown of bacteria colonies in post test.

3. Findings and Results
The various stages of screening under take to obtained cellulase probiotic. Destruction of the abdomen was carried out by separating the superior parts, so the pure isolate was obtained without contamination from the other body parts [20]. Table 1 showed that bacterial isolates were selected from the results of the dilution, and screening assay: cellulolytic/cellulase enzyme activity test, pathogenicity test, human stomach acid and salt osmotic resistance test.
### Table 1. Screening assays results: The selected bacteria isolates potentially probiotics in 10^{-4} dilution/ isolates 4.1, 4.1’ dan 4.3

| No | Isolates | Cellulolytic Test | Human stomach acid resistance test | Pathogenicity test | Salt osmotic concentration resistance test |
|----|----------|--------------------|-----------------------------------|--------------------|------------------------------------------|
| 1  | 4.1      | +                  | +                                 | +                  | +                                        |
| 2  | 4.1’     | +                  | +                                 | -                  | +                                        |
| 3  | 4.2      | -                  | -                                 | -                  | -                                        |
| 4  | 4.3      | +                  | +                                 | -                  | +                                        |
| 5  | 4.5      | -                  | -                                 | -                  | -                                        |
| 6  | 5.1      | -                  | -                                 | -                  | -                                        |
| 7  | 5.2      | -                  | -                                 | -                  | -                                        |
| 8  | 5.3      | -                  | -                                 | -                  | -                                        |
| 9  | 5.4      | -                  | -                                 | -                  | -                                        |

(+) : Positive : (qualified on test)
(-) : Negative : (not qualified on test)

#### 3.1. Cellulolytic Test

The screening was carried out to test bacterial activity of cellulose. Figure 1 showed that isolate of 4.3, 4.1 and 4.1 ‘(isolates were taken in the same dilution) released the clear area faded around bacterial colonies. This result can be analyzed that the isolate selected can break down the cellulose medium into glucose by screening test using Congo red medium and CMC. Congo red as an indicator for cellulolytic enzyme activity released by bacteria [21]. The ability of the cellulolytic activity that broke down into glucose, derived from the analysis that cellulase bacteria in termite colon was useful for obtained the glucose in the termite's body from consumed of wood [22]. The endurance of 4.1, 4.1 ’and 4.3 bacteria in the acidic medium also showed that bacteria potentially qualified the stomach acid, that was as a probiotic candidate. Cellulolytic enzymes to broke down cellulose, must be in acidic conditions to be able to hydrolyze the bonding cellulose polymer between glucose [23].

![Figure 1](image1.png)

**Figure 1.** Results of screening bacterial cellulase and acid resistance: a-isolates 4.3; b-isolates 4.1; c-isolates 4.1’ (on a 10^{-4} dilution).

#### 3.2. Pathogenicity Test

Blood agar base medium and sterile human blood type of O are used to detect the pathogenicity activities of the bacteria by hemolysis asssaelement [24]. The cellulase bacteria strains were streaked on blood agar plates that containing 5% human blood and 2.5% sodium chloride (NaCl), the plates were incubated for 48 hours at 37°C [25]. It was carried out to determined the potential in terms of safety for the human body as probiotics. Figure 2 showed the results that isolate passed through cellulolytic screening 4.1’ had the less of hemolysis, β hemolysis. But it had less of ability to lyse the red blood cells with the
certain amount. On the other hand, cellulase bacteria of 4.3 and 4.1 showed the positive result with non pathogenic activity (γ hemolysis). Bacterial isolates of 4.3 and 4.1 showed that there were not the damage of the blood medium, there were not discoloration or the formation of the clear zones around the colonies. From the results, they were potentially non pathogenic for probiotic cellulase candidates, as in [17]. The γ hemolysis strains are the most promising probiotic candidates, and should not be either pathogenic or able of producing toxic substances that may harm for the body [25].

3.3. Human Stomach Acid and Salt Osmotic Concentration Resistance Test
Probiotic should be able to lived in the digestive conditions [26]. Among the conditions in the digestion was the stomach acid pH 2-2.5 and the osmotic salt (6.5% NaCl) [27]. The resistance was necessary to bacteria kept working according to the function [28]. Table 2 showed the test results that Isolates of 4.1 and 4.3 can grow from the pH 2.5 treatment and with 3.5% and 6.5% salt osmotic concentrations for 2 hours (2H) and 4 hours (4H) digestive incubation period. Both of the isolates were tolerant with the high osmotic concentration of salt (NaCl 3.5% and 6.5%), after the treatment and incubation for 24 hours. Isolate grown in testing media by streaking, after incubation time in human gut condition. It can be analyzed that both of bacterial isolates can survive in the digestive conditions than other acids, such as NaCl osmotic concentration as Menconil et al. [18]. All of bacteria strains had a similar behaviour to different pH exhibiting no growth at pH 1-3, but grow at pH higher than 3. It has been estimated that the survival rate of traditional probiotics in the host’s gut is only 20-40%, being gastric acidity one of the main obstacles. Other properties, to be considered are tolerance to salt and different pH values as well as the in vitro ability to colonize the host gastrointestinal tract [29].

The availability of human ability to digest polymer cellulose to glucose from termite gut microbes was the chance to process the grass as a new staple food, without concerning the agriculture result. Through cellulase probiotic, whatever the materials were high in cellulose, it can be directly consumed and digested by humans’ body with the help of termite gut microbes.

| No | Isolates | Stomach acid resistance test | Salt osmotic concentration resistance test |
|----|---------|-------------------------------|-------------------------------------------|
|    |         | pH 7 2H | pH 2-2.5 4H | 3.5% NaCl 2H | 6.5% NaCl 2H |
| 1  | 4.1     | +       | +           | +            | +            |
| 2  | 4.1’    | +       | +           | +            | +            |
| 3  | 4.3     | +       | +           | -            | +            |

(+): Positive: (tolerant on treatment)
(-): Negative: (not tolerant on treatment)
4. Conclusion
There are isolated termite gut microbes (bacteria) analyzed can be used in human gut as probiotic candidate to digest cellulose to glucose by in vitro testing. It can be sustainability research for in vivo testing, before the termite gut microbes selected can be consumed by human in the future for probiotic in the global food scarcity to eat cellulose material, like grasses and sawdust with high cellulose.

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