

Optimization of Mannanase Enzymes Production from *Bacillus cereus* V9 Using Local Mannan Substrate

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ABSTRACT

Bacillus cereus V9 is a mannanolytic bacteria capable of producing a mannanase enzyme of 29.5 IU / mL with a substrate of Locust bean gum. The use of Locust bean gum as a substrate in the production of the enzyme mannanase is not recommended because it is expensive so that the enzyme produced will be expensive. The use of local mannan substrates such as porang, coconut pulp, coconut cake, and palm kernel meal has the opportunity to replace Locust bean gum because the mannan content is almost equal to the content of the mannan Locust bean gum so the resulting enzyme is cheaper. This study aims to determine the best local mannan biomass substrate as a substitute for Locust bean gum for the production of the mannanase enzyme from *Bacillus cereus* V9 bacteria. This study consisted of two stages where the first stage was the optimization of the production of mannanase enzymes in various alternative substrates for Locust bean gum, then the second stage was the production of the enzyme mannanase from the selected substrate as a substitute for Locust bean gum. Simple data analysis is based on calculating the number and average of each observation from 4 replications and the data is displayed in the form of a descriptive narrative. The results showed that *Bacillus cereus* V9 could grow on all local mannan media and the highest was on palm kernel meal media. The growth of *Bacillus cereus* V9 at a substrate concentration of 1% palm kernel meal did not differ from a concentration of 1.5% and 2%. The activity of the mannanase enzyme *Bacillus cereus* V9 in palm kernel meal media was higher when compared to porang, coconut cake, and coconut pulp. This study concludes that palm kernel meal can be used as a 1% substrate as a substitute for Locust bean gum in the production of the enzyme mannanase from *Bacillus cereus* V9.

Keywords: palm kernel meal, porang, coconut meal, coconut cake, Locus bean gum, mannanase.

1. INTRODUCTION

The mannanase enzyme is an enzyme that plays an important role in breaking down mannan into mannose and mannan-oligosaccharide (MOS) [1]. Mannanase enzymes are widely used in the paper industry, as a hydrolytic agent in the detergent industry, in the hydrolysis of coffee extracts, in the improvement of animal feed, like a fish feed additive, as a mucus control agent, and in the pharmaceutical industry [2]. Several research results show that the addition of the mannanase enzyme either directly or through pre-treatment can reduce the crude fiber content of palm kernel cake so that it can improve its quality as animal feed [3][4][5][6][7].

Mannanase enzymes can be produced from bacteria, plants, fungi, and invertebrates⁴. The production of mannanase enzymes from microorganisms is superior because the production process is fast, the production conditions are more controlled, and the quality is uniform

[8]. One of the microorganisms that can produce the mannanase enzyme is the bacterium *Bacillus cereus* V9 [9].

Bacillus cereus V9 is a mannanolytic bacteria isolated from the digestive tract of termites which is capable of producing mannanase enzymes on Locust bean gum (LBG) substrate with the enzyme activity of 29.5 U/mL⁹. According to Sumardi et al.[10] the production of the enzyme mannanase usually uses a pure mannan substrate as a carbon source such as LBG. The use of LBG as a substrate requires a large amount of money because it is expensive and difficult to obtain in the market. This condition provides an opportunity for the local mannan substrate to be used as the main carbon source to replace LBG in the production of mannanase enzymes so that the production costs will be cheaper.

In Indonesia, there are various types of plants and by-products from agroindustry which contain quite high levels of mannan, which can be used as a carbon source

in the production of the enzyme mannanase. Porang, coconut cake, coconut pulp, and palm kernel meal are the by-products of agro-industry which contain high enough mannan to replace LBG as a substrate for microbial growth in producing enzymes. LBG contains 80% mannan while 78% palm kernel meal, 69.36% porang, 61% coconut cake, and 61.8% coconut pulp [11][1].

Optimization of enzyme production from microbes is largely determined by the type of substrate that will be used as a carbon source for growth and the length of incubation for microbes to produce enzymes [12]. Meanwhile, Sumardi et al.¹⁰ stated that the mannanase enzyme activity from bacteria would be optimized by paying attention to the concentration of the substrate used, the pH, and temperature of the media during the microbial fermentation process [13]. Therefore, for optimization in the production of the mannanase enzyme from *Bacillus cereus* V9 using a local mannan substrate, it is necessary to pay attention to the factors mentioned above so that a more economical and practical enzyme will be produced.

2. RESEARCH METHOD

2.1 Isolate Rejuvenation

The rejuvenation of *Bacillus cereus* V9 bacteria was carried out by growing it in Nutrient Broth (NB) liquid media by adding 0.5% locust bean gum. Subsequently, it was incubated in a shaker incubator for 48 hours with a rocking speed of 130 rpm at room temperature. The grown bacteria were transferred to solid media containing 0.5% locust bean gum and 1.5% bacto agar, 0.05% yeast extract, 0.075% peptone, and the minerals Mendels and Sternberg[14]. Then it was incubated for 24 hours and then the bacteria were ready to be used as stock for multiplying microbes.

2.2 Selection of Local Mannan Substrate as a Substitute for Pure Mannan Substrate (LBG)

The media used for the production of the mannanase enzyme from *Bacillus cereus* V9 was a liquid medium containing 1.5% bacto agar, 0.05% yeast extract, 0.075% peptone, 0.5% for each substrate (Locust bean gum (LBG) as control, porang (PR), coconut pulp (CP), coconut meal (CM) and palm kernel meal(PKM)) and minerals Mendel and Stenberg¹⁴. The liquid media was made into two parts, namely for the pre-culture media as much as 30 mL of 100 mL natural erlemayer and 300 mL of culture media which were placed in the 500 mL erlemayer. All materials were dissolved in distilled water on each layer according to the substrate used and then sterilized in autoclave at a temperature of 121°C and a pressure of 1 atm for 15 minutes.

The rejuvenated bacteria were inoculated aseptically on pre-culture media for 3 ose then incubated in a shaker

incubator for 24 hours. The precultures that had been grown with *Bacillus cereus* V9 were then transferred to culture media aseptically and again incubated for 120 hours. Harvesting of bacteria was carried out every 8 hours as much as 4 mL where 2 mL was used to measure microbial cell growth while 2 mL was used to measure the activity of the crude extract enzyme. Mannanase enzyme crude extract was obtained from cell culture extraction by centrifugation at a speed of 12,000 rpm for 15 minutes. Then the supernatant obtained is ready to be used for measuring the activity of the mannanase enzyme.

The growth patterns of bacteria were measured by reading at a wavelength of 660 nM. Whereas the measurement of the activity of the mannanase enzyme was based on Meryandani et al [15], by incubating 0.5 mL of mannanase enzyme crude extract solution with 0.5% (w/v) LBG substrate in a buffer phosphate solution pH 6 at 30°C for 30 minutes. Then the reaction is stopped by immersing the test tube in boiling water (temperature 90°C - 100°C) for 20 minutes. Reducing sugar released was measured using the DNS method [16] then the color formed was measured using a spectrophotometer at a wavelength of 540 nM. One unit of the mannanase enzyme is defined as the amount of the mannanase enzyme which can produce 1 μ mol of reducing sugar (mannose base) for 1 minute. At this stage, the pattern of bacterial growth and the highest mannanase enzyme activity will be obtained on the selected substrate and for further optimization of the enzyme production on the selected substrate.

2.3 Optimization of Selected Substrate Concentrations and Incubation Time.

After obtaining the selected substrate to replace LBG in the production of the mannanase enzyme from *Bacillus cereus* V9, then the substrate concentration was optimized with 4 concentration levels, namely 0.5%, 1%, 1.5%, and 2%. The liquid media used is the same as the media for substrate selection, but the substrate used is the substrate chosen to replace LBG. The procedure for measuring microbial cell growth and enzyme activity is also the same as for the determination of local mannan substrate selection as a substitute for pure mannan substrate (LBG). At this stage, the optimal concentration and incubation time will be obtained in the production of the enzyme mannanase using the selected substrate.

2.5 Data analysis

Data collection in the first stage of research is based on calculating the number and average of each observation from 4 replications and the data is displayed in the form of a descriptive narrative [17].

3. RESULTS AND DISCUSSION

3.1 Selection of local mannan substrate as a substitute for LBG in the production of the enzyme mannanase

The rejuvenated *Bacillus cereus* V9 bacteria were then grown on various local mannan substrates showing different growth patterns from the locust bean gum substrate. The growth pattern of *Bacillus cereus* V9 in various substrates can be seen in Figure 1.

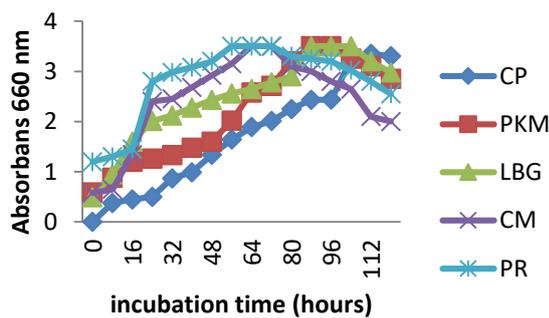


Figure 1. The Growth of *Bacillus cereus* V9 cells on various local mannan substrates.

The growth curve of *Bacillus cereus* V9 cells on porang substrate reached the peak point of cell growth faster than other local mannan substrates. Then followed by the substrate of coconut cake, locust bean gum, palm kernel meal, and coconut dregs. The results of this study indicate that the use of local mannan substrate as a substitute for LBG substrate can be used as a source of nutrition for the *Bacillus cereus* V9 bacteria. One of the essential nutrients for bacterial cell growth is carbon which will be used for growth and replication processes. According to Greg and Ricahad¹⁸ that bacteria will obtain carbon from the enzymatic breakdown of complex organic molecules such as carbohydrates.

The substrate is very important for cells for biomass growth, cell maintenance and to produce products from its breakdown. The type of substrate used for bacterial growth is usually adjusted to the type of enzyme to be produced. The incubation time in substrate fermentation shows a relationship between the use of the substrate and the production of the enzymes produced, where the production of enzymes decreases in line with the reduced availability of nutrients in the substrate [19]. Mannanase is the primary metabolite needed to break down the mannan contained in the substrate used by bacteria for their growth. In the initial phase, it was seen that *Bacillus cereus* V9 had not produced much mannanase because they still used other sources of nutrition from yeast extract as a nitrogen source. Bacteria will take advantage of the mannan present in the substrate after entering the

exponential phase which is indicated by an increase in the production of the enzyme mannanase. According to Chen²⁰ that yeast extract is generally used as a stimulator for microbial growth.

Mannan is a polysaccharide compound or complex carbohydrate that is difficult for cells to digest directly. Mannan will be degraded into simple sugars such as mannose and oligosaccharides by bacteria by secreting a mannanase enzyme. The activity of the mannanase enzyme from *Bacillus cereus* V9 on various local mannan substrates can be seen in Figure 2.

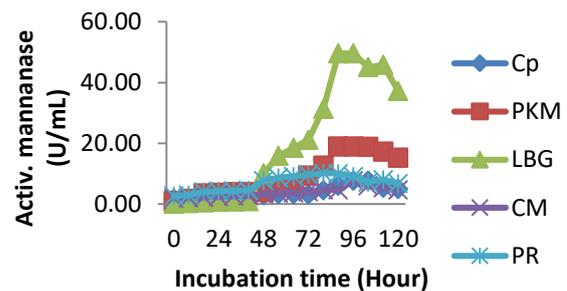


Figure 2. The activity of the mannanase *Bacillus cereus* V9 on the local mannanase substrate

The activity of the *Bacillus cereus* V9 mannanase enzyme on palm kernel cake media showed the highest activity when compared to other local mannan media. The activity of the mannanase enzyme *Bacillus cereus* V9 in palm kernel meal media reached 18,95 U / mL from the 88th hour to the 104th hour. The high mannan content in palm kernel meal which reaches 30-70%^{21,22,23} causes bacteria to produce mannanase enzymes in large quantities to degrade mannan being a much simpler compound. Chuan et al.[24], reported that palm kernel meal can be used as a substrate for the production of the enzyme mannanase from *Bacillus subtilis* ATCC3366 with a mannanase activity of 8 U / mL. Norizan et al.[25], also reported the use of palm kernel meal as a substrate to produce the enzyme mannanase produced from *Bacillus subtilis* ATCC11774. Thus, the results of this study indicate that palm kernel meal has a great opportunity as a substrate to replace locust bean gum for the production of the enzyme mannanase by *Bacillus cereus* V9.

3.2 Optimization of Palm Kernel Meal Concentration as Substrate

Based on the ability of *Bacillus cereus* V9 to produce mannanase enzymes on various local mannan substrates, palm kernel meal was chosen as an alternative substrate to replace locust bean gum. Although the mannanase enzyme activity of *Bacillus cereus* V9 was not as good as that of the locust bean gum medium, it had better activity than other local mannan substrates. Optimization of

mannanase production from *Bacillus cereus* V9 using mannan substrate increased with increasing concentration of palm kernel meal. The growth of *Bacillus cereus* V9 bacteria at various concentrations of palm kernel meal substrate can be seen in Figure 3.

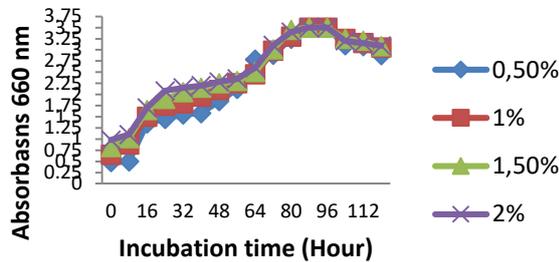


Figure 3. Growth of *Bacillus cereus* V9 at various concentrations of Palm Kerneal Meal

The growth of *Bacillus cereus* V9 at various concentrations of palm kernel meal substrate showed the same growth pattern. The bacterial growth rate reached its optimum point at the 88th hour to the 96th hour then decreased until the 120th hour. The bacterial growth curves formed to coincide with each other at 56 to 120 hours. Nevertheless, the results of the analysis of the mannanase enzyme activity from various substrate concentrations showed that the 1% palm kernel meal substrate concentration had a higher enzyme activity value than other concentrations, reaching 30.25 U / mL (Figure 4).

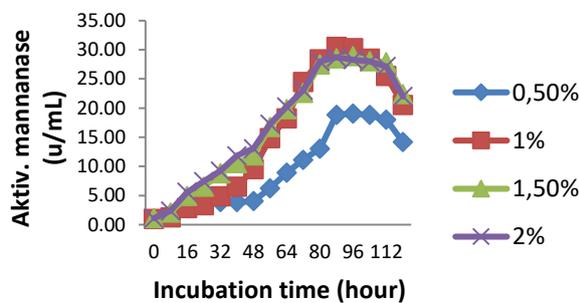


Figure 4. The activity of the mannanase enzyme *Bacillus cereus* V9 at various PKM concentrations

The results of the analysis of the mannanase enzyme activity showed that at the optimization stage the substrate concentration showed that the PKM substrate concentration was 1% higher than the concentration of 0.5%, 1.5%, and 2%. The activity value of the mannanase enzyme at 1% substrate concentration at the 88th hour reached 30.25 U / mL while for concentrations of 0.5%, 1.5% and 2% were 18.85 U / mL, 28.50 U / mL and 28.69 U / mL. At a substrate concentration of 0.5%, it was seen that the enzyme reached its peak production at the 88th hour and almost simultaneously with other substrate concentrations, but had a lower value. Whereas at a higher substrate concentration (2%), the mannanase

activity value was lower than the 1% substrate concentration. A high substrate concentration will cause the solubility of oxygen in the water to be low because the viscosity increases, thus inhibiting bacterial growth. High viscosity will reduce the ability of the enzyme to break the bonds of the components contained in the substrate [26] Meanwhile, according to Madigan et al., [27] that the low solubility of oxygen in water and replacement of oxygen through slow diffusion are growth-inhibiting factors. aerobic living bacteria. The results of this study indicate that the most appropriate concentration for the production of the enzyme mannanase from *Bacillus cereus* V9 with palm kernel meal substrate is at a substrate concentration of 1%.

4. CONCLUSION

This study concludes that palm kernel meal can be used as a 1% substrate as a substitute for locust bean gum in the production of the enzyme mannanase from *Bacillus cereus* V9.

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