The effect of Different Media Content on Protease Activity Bacillus subtilis

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ABSTRACT

Enzymes were protein molecules that synthesized cells to accelerate biochemical reactions. One of the microorganisms that produce a protease was Bacillus subtilis. B. subtilis used in this research consisted of isolate 1 and isolate 2. Methods used include the qualitative assay of protease activity through the clear zone on skim milk agar and calculation of protease activity on production media such as NB (Nutrient Broth) and TSB (Tryptic Soy Broth). Based on the results revealed that the clear zone diameter of isolate 2 better than the isolate 1 after 24 hours and 48 hours incubation periods. It showed both isolates having the possibility in different strains but within a species. The highest protease activity calculation obtained from TSB production media respectively 0.14 Unit/ml unit and 0.12 Unit/ml.

Keywords: B. subtilis, clear zone, protease, TSB

INTRODUCTION

Enzymes are protein molecules that synthesized cells to accelerate biochemical reactions [1]. The specificity of the enzyme is affected by the substrate being used, such as protease enzyme will only hydrolyze proteins into peptides and amino acids. Proteases can be produced by plants, animals, and microorganisms [2]. This enzyme is widely used in industry, such as detergents, food industry, industrial non-food and biological catalysts [3].

One of the microorganisms that produce a protease is Bacillus subtilis. Bacillus subtilis is a Gram-positive bacteria that are saprophyte and can be found in soil, air, and plants that have been weathered. These bacteria are non-pathogenic, grow quickly, and easily upgraded enzyme results through setting growth conditions and genetic engineering [4]. Therefore, the research analyzed the effect of using different production media content against B. subtilis protease activity.

METHODS

Qualitative Assay of Protease Activity Bacillus subtilis. The microorganisms used in this research were Bacillus subtilis consisted of isolate 1 and isolate 2. Both of these isolates were grown in 100 ml of skim milk agar [skim milk (10%) and agar (2%)] at a temperature of 37°C for 48 hours. Protease activity was characterized by the formation of a clear zone around the colony.

Assay of Proteolytic Activity of Bacillus subtilis. Isolates of Bacillus subtilis which has the best proteolytic index were grown in 100 ml of NB (Nutrient Broth) and TSB (Tryptic Soy Broth) at 37°C with 150 rpm for 48 hours [5]. After 48 hours performed centrifugation at 10,000 rpm with a temperature of 4°C for 10 minutes. Supernatant obtained was used as a crude enzyme [6]. Measurement of protease activity carried out by the mixing 200 µL of casein 500 ppm, 300 µL of phosphate buffer pH 7, and 100 µL of an enzyme (crude enzyme) and incubated in temperature of 37°C for 60 minutes. Then added 4% TCA (Trichloro Acetic Acid) and allowed to stand for 30 minutes at a temperature of 27°C (room temperature). After that was performed centrifugation at 4000 rpm for 10 mins. 100 µL of the supernatant was taken and diluted to 5 times the volume of the sample by using...
phosphate buffer and measured absorbance value at tyrosine λ maximum of 275 nm. The Calculations based on the protease activity to hydrolyze casein to be tyrosine. Then tyrosine released by the enzyme was calculated from standard tyrosine curve (concentration of 10-100 ppm).[3].

RESULTS AND DISCUSSION

Both isolates of B. subtilis were grown on skim milk agar to determine the proteolytic activity. Proteolytic activity was shown by the ability of bacteria to hydrolyze a substrate and to result in the clear zone around the colonies. Isolate 2 produced the clear zone diameter better than the isolates after 24 hours and 48 hours incubation periods (Figure 1). It showed that both isolates had the possibility in different strains but within a species. Clear zone formed around the colonies of B. subtilis showed that protease hydrolyze the casein molecules on the substrate to be peptides (simple proteins) and amino acids [3].

One of the factors that influenced the proteolytic activity of bacteria was the content of the media that used as nutritional sources. Skim milk agar contained many nutrients such as casein, calcium, potassium, magnesium, phosphorus and others. The content of casein was phosphoprotein that can bind with calcium to formed calcium calcinate. The color of the suspension was white, so it was more easily to observed in the solid media culture [7].

Protease activity from both isolates of B. subtilis were higher on TSB production media than NB production media (Figure 3). The maximum protease activity produced respectively 0.14 units / ml and 0.12 units / ml on TSB production media and the lowest activity produced respectively 0.036 units / ml and 0.044 units / ml on NB production media. It showed that the content of substrate influenced the bacterial protease activity. TSB production media containing 17 g tryptone, 3 g of soy peptone, 2.5 g of dextrose, 5 g NaCl, and 2.5 g $K_2HPO_4$ while NB production media containing 5 g of peptone and 3 g of beef extract. The content of TSB production media was more complex than NB production media so that the result more better.
by giving specific substrate (inducible) in the production of media content [8]. The content of nutrients in the media was one of the variables that affected the submerged fermentation of enzymes production [9]. Moreover, the higher concentration of substrate also affected the activity of the enzyme to accelerate the reaction of the enzyme-bound substrate [10].

CONCLUSION

Both isolates of *Bacillus subtilis* had different clear zone diameter in a qualitative protease activity assay so that there was possibility both isolates had different strains within a species. The highest protease activity obtained from TSB production media respectively by 0.14 units /ml and 0.12 U / ml.

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REFERENCES