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MAKALAH POSTER

# Fibrinolytic Activity of Bacterial Isolates from The Papuma Beach on Jember District

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# Biodiversity and Biotechnology for Human Life

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# Aktivitas Fibrinolitik Isolat Bakteri dari Perairan Pantai Papuma Kabupaten Jember
# Fibrinolytic Activity of Bacterial Isolates from The Papuma Beach on Jember District

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***Abstrac***

*Papuma Beach has a high bacterial diversity. The results of the previous research were obtained 23 bacterial isolates. A potential that can be exploited was the ability to produce a fibrinolytic enzyme that can be used to solve various problems of thrombosis (myocardial infarction). The purpose of this research was to examine proteolytic and fibrinolytic activities of bacterial isolates from Papuma beach. Proteolytic activity test performed using skim milk agar (SMA) media on 23 bacteria isolate and fibrinolytic activity test using Fibrin Plate Assay method on isolate that are known to have proteolytic activity. Eleven bacterial isolates have proteolytic activity and three bacterial isolates have fibrinolytic activity. Bacterial isolates WU 021012* has the highest proteolytic index i.e. 4.3. Bacterial isolates that has fibrinolytic activity i.e WU 021012*, WU 021001* and WU 021055* with fibrinolytic index 7.01, 6.95, and 5.86 ,respectively.*

***Keywords:*** *fibrinolytic, bacterial isolates, Papuma Beach on Jember District, proteolytic.*

## Introduction

Cardiovascular diseases such as acute myocardial infarction are primary causes of death throughout the world (Simkhada *et al*., 2010). According to World Health Organization (WHO) in 2004, 12% of the total mortality rate in the word is caused by cardiovascular deases (WHO, 2008). Myocardial infarction is a disease caused by the formation of a blood clot (fibrin) in the circulatory system that can cause vascular blockage leading to serious consequences including death (Banerjee *et al*., 2003). In balanced hemostatic system, blood clots in the circulatory are hydrolyzed by plasmin. But in unbalance condition, the clots can not be hydrolyzed, and thus thrombosis occurs. These abnormalities can be treated with thrombolytic agents, such as Urokinase-type plasminogen activator and tissue plasminogen activator (t-PA) (Simkhada *et al*., 2010). Although, these are still widely used, their

expensive price and undesirable side effect, such as risk for internal hemorrhage within the intestinal tract, prompt researcher to search for cheaper and safer resources. Therefore microbial fibrinolytic enzyme become more interest in this decades (Peng *et al.*, 2005). Streptokinase from *Streptococcus hemolyticus* and staphylokinase from *Streptococcus aureus* were earlier proved to be effective in thrombolityc therapy (Collen and Lijnen, 2004). Over the year, more fibrinolytic enzyme from various microbes have been discovered, such as *Bacillus subtilis* BK-17 (Jeong *et al*., 2001), *Bacillus subtilis* AI (Jeong *et al*., 2004), *Bacillus subtilis* 168 (Kho *et al*., 2005), *Bacillus sp* B1 (Sanusi dan Jamaluddin, 2012), *Bacillus amyloliquefaciens* CH51 (Kim *et al*., 2009), *Bacillus subtilis* Natto B-12 (Wang *et al*., 2009), *Streptomyces megasporus* SD5 (Chite dan Dey, 2000), *Streptomyces lividens* (Pimienta *et al*., 2007), and *Streptomyces sp*. CS684 (Simkhada *et al*., 2010). The previous study were obtained 23 bacterial isolates from Papuma beach in Jember, East Java, Indonesia, with 11 isolates are proteolitic. Three isolates from proteolytic isolates are fibrinolytic, include WU 021012*, WU 021001*, and WU 021055* with fibrinolytic index respectively 7,01, 6,95 dan 5, 86.

## Materials and Methods

a. Test Microorganism

   The tested microorganism, WU 021012*, WU 021001*, and WU 021055*, were collections of Microbial Laboratory of The Mathematics and Natural Sciences Faculty, Jember University. Three bacteria isolates were fibrinolytic activities tested using fibrin plate assay method.

b. Growth curve of bacteria isolates WU 021012*

   Growth curve was made by the direct method, which calculates with microscope. The culture then was incubated at 37° C with time interval 4 hour for 36 hours.

c. Cell-free supernatant (CFS) production and CFS Precipitation

   Single colony of bacteria isolate WU 021012* was cultivated in LB liquid medium at 37 ºC with shaking (as a starter) for 12 hours (overnight). Five percents (v/v) starter was grown in LB liquid medium at 37 ºC with shaking. CFS production carried out in six times, that was at hours 8, 12, 24, 36, 48, and 60. In every hour the bacterial culture was taken and centrifuged 10.000 rpm, 4 ºC for 15 minutes. The supernatant is CFS (8, 12,

24, 36, 48, 60). The CFS was further concentrated by cold acetone precipitation with a ratio of cold acetone and CFS in 5:1, then it was incubated at -20 ºC for 1-2 hours, and centrifuged 10,000 rpm, 4 ºC for 15 minutes. The acetone pellet (protein precipitate) was redissolved in a small volume of PBS pH 7,4.

d. Assay of Fibrinolytic Activity

Fibrinolytic activity was determined using fibrin plate assay method. Fibrin plates were prepared by using 5-cm-diameter petri dish. Fibrinogen (0,1%; 10 ml) in phosphate buffered saline was clotted with 0,2 ml of bovine thrombin (10 National Institutes of Health U/ml) in 0,5 M $CaCl_2$ (Lottenberg et al., 1987) and 1,2% agarose. 10 µl CFS and protein precipitate respectively dropped into wells in the fibrin media, and the plates were incubated for 15 h at 37° C, and the degree of hydrolysis was scored by measuring the area of the zone of clearing from the underside of the plate. Each experiment was done in duplicate and then performed by LSD statistical tests to determine the time production with the largest fibrinolytic activity.

## Result and Discussion

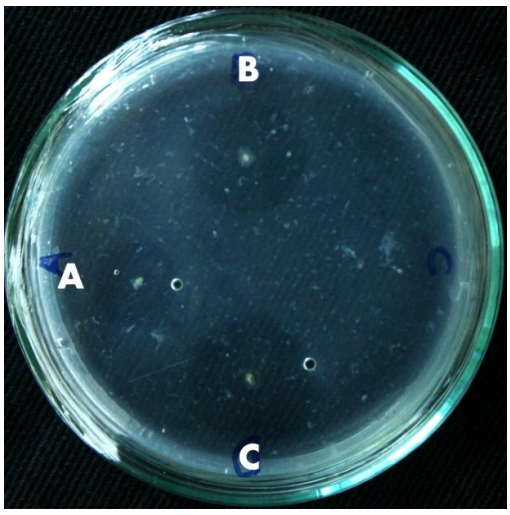


Figure 1. Clear zone that shows fibrinolytic activity of bacterial isolates a) WU 021055*; b) WU 021001*; c) WU 021012* in fibrin media, with respectively 5.86, 6.95, and 7.01.

Proteolytic activity screening results were conducted on 23 bacteria isolates from Papuma Beach Jember in skim milk agar media showed that 11 isolates had proteolytic activity, while the 12 isolates are non-proteolytic (Setiawan, 2010). Proteolytic bacterial isolates showed that the bacteria were able to produce extracellular protease enzyme. Volk

and Wheeler (1993) states that the proteolytic bacteria are bacteria that were able to produce extracellular protease enzyme. Extracellular protease enzyme is one of the enzymes that degrade proteins, that produced in the cell and released to the outside of the cell. Non-proteolytic bacterial isolates have protease enzyme in the cell but not released.

From eleven bacterial isolates that are proteolytic, only three bacterial isolates had fibrinolytic activity. The three bacterial isolates were WU 021012*, WU 021001*, and WU 021055* with fibrinolytic index respectively 7.01, 6.95 and 5.86 (Figure 1). Determination of the fibrinolytic activity using fibrin plate assay method (Lottenberg et al., 1987). Fibrinolytic activity were determined by the ability of the enzyme that can hydrolyze fibrin substrate, indicated by formation of a clear zone around the bacterial colonies (figure 1). Milner and Makise (2002) claimed that the formation of a clear zone width and clearly indicates more fibrin hydrolyzed by fibrinolytic enzymes.

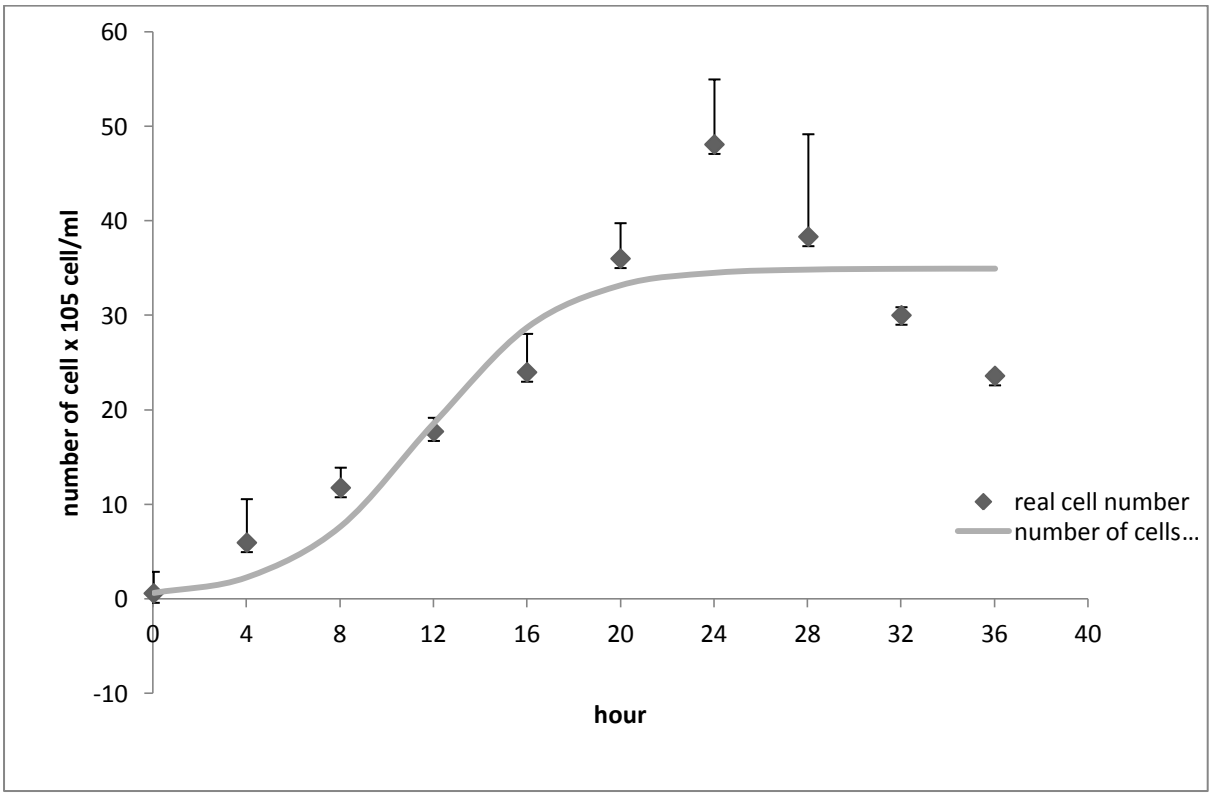


Figure 2. Growth curve bacterial isolates WU 021012* for 36 hours

Bacterial isolates WU 021012* were determined growth curve to find out exponential phase (log phase). The growth curve of the bacterial isolates can be seen in Figure 2. Adaptation phase of the bacterial isolates WU 021012* is from 0 to 6th hour. This adaptation

time is short, because the media was used as the inoculum (starter) for the initial growth is same with bacterial culture media. According to Volk and Wheeler (1993), the adaptation phase occurs for one hour to several hours depending on the type of bacteria, age of culture, and the nutrients contained in the media.

After the bacterial cells had early growth phase, bacterial cells enter the exponential phase. In this phase, cells start to divide with a very fast pace and it can be said exponential phase of bacteria. Exponential phase of bacterial isolates WU 021012* starting at the 8th until the 20th hour. According Kosin and Son (2010), the bacteria energy required in this phase is higher than in other phases and in this phase many cells produce enzymes and substances of primary metabolites that needed to growth and development. Senjarini (2007) states that highly rapid growth of bacteria in the exponential phase indicates that in these phase, bacteria has high activity and the best metabolism. It is used as a basis to determined the crude extract protein or cell-free supernatant (CFS) production time.

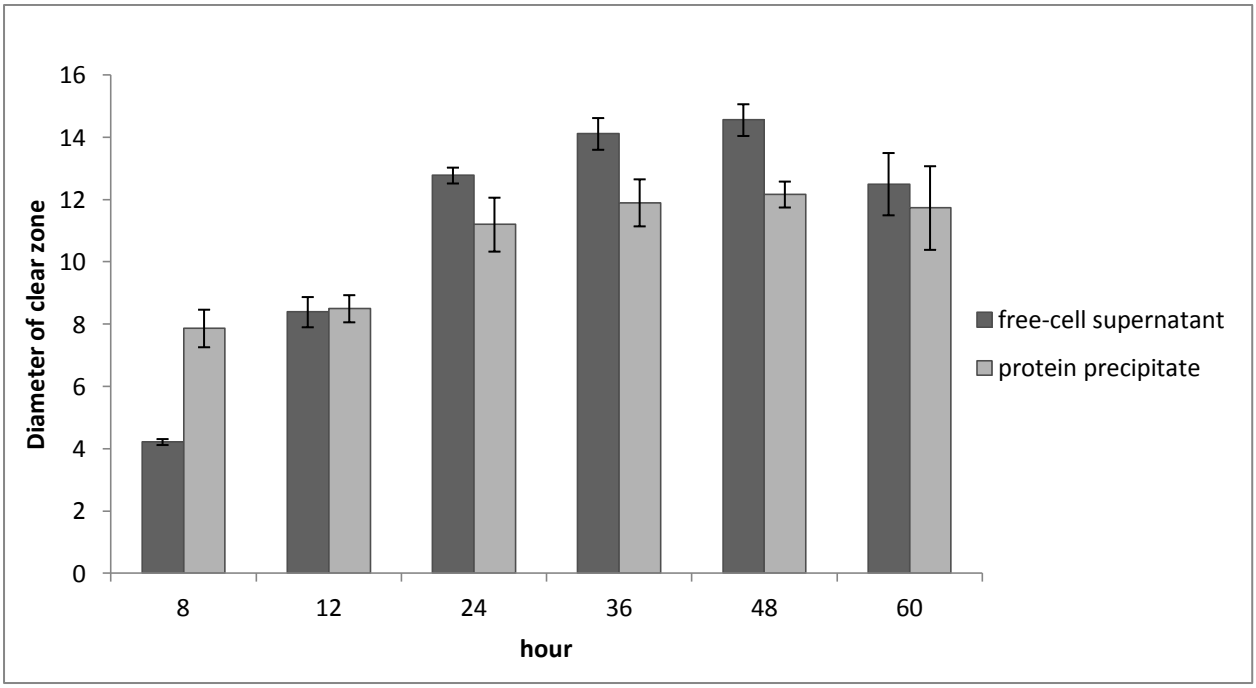


Figure 3. Enzyme activity of cell-free supernatant and protein precipitate 8, 12, 24, 36, 48, and 60.

In this research, crude extract protein or cell-free supernatant production carried out at hours 8, 12, 24, 36, 48 and 60. The purpose is to determine the fibrinolytic activity of each production time, so we can determined the production time with the largest fibrinolytic activity. The result of the fibrinolytic assay CFS (8, 12, 24, 36, 48, 60) can be seen in figure

3. Based on obtained results indicate that CFS which has a high fibrinolytic activity are CFS 24, 36, 48, and 60. Based on the results of LSD statistical tests, fibrinolytic activity CFS 36 and CFS 48 did not have a significant differences, so the optimum time for the CFS production is at 36 hour. The CFS was further concentrated by cold acetone precipitation, and then fibrinolytic activity of the protein precipitates were obtain to determine acetone effect on fibrinolytic activity. The result of the fibrinolytic assay of protein precipitates can be seen in figure 3. Based on Obtained results indicate that all of the protein precipitates have fibrinolytic activity. Therefore we can conclude that the acetone does not affect fibrinolytic activity.

## Conclusion and Outlook

From eleven bacterial isolates that are proteolytic, only three bacterial isolates had fibrinolytic activity. The three bacterial isolates were WU 021012*, WU 021001*, and WU 021055* with fibrinolytic index respectively 7,01, 6,95 dan 5, 86. Crude extract protein or CFS production by bacterial isolates WU 021012* at hours 8, 12, 24, 36, 48, and 60, which has the highest fibrinolytic activity is CFS 36. Protein precipitates or the results of CFS precipitation with cold acetone at all the time production has fibrinolytic activity, so we can be conclude that the acetone does not affect on fibrinolytic activity.

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