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The Effects of Carbon Source and Temperature on the Growth and Granaticin Production in Streptomyces Thermoviolaceus.

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Abstract

Streptomyces thermoviolaceus is gram positive, aerobic, filamentous shape bactrium which usually can be found in soil. It is a useful model that can be researched for second metabolism. There are many factors may affect the production of Streptomyces thermoviolaceus such as carbon source, pH and temperature. Streptomyces thermoviolaceus is used industrially as a granaticin producer. In order to determine the optimal conditions of production of granaticin, the three different carbon source which are glutamate, proline and glucose and three different temperatures which are 40°C, 45°C and 50°C were set as cultural conditions. After the Quantitative measurements of the antibiotic showed that glutamate was the best carbon source at 45 °C for secondary metabolism. Meanwhile, the highest biomass was obtained at 50°C and lowest at 45 °C.

1. Introduction

Streptomyces thermoviolaceus is a kind of gram positive bacterium usually can be found in multiple of soil containing water and plants which is provided characteristics for instance aerobic, filamentous form (Hasani, Kariminik, and Issazadeh, 2014). It belongs to the Actinomycetes group and shares many features with fungi (Iqbal et al., 1994). Hasani has referred that 80% of antibiotics are derived from Streptomyces sp (Hasani, Kariminik, and Issazadeh, 2014). In nowadays, although the discovery of antibiotics is success, the mortality of infectious diseases still is one of the highest. The microbial resistance plays an important role to resist the antibiotics due to genetic changes. Therefore, Streptomyces thermoviolaceus is a useful model that can be researched for second metabolism. Streptomyces thermoviolaceus can produce second metabolites for example antibiotics.

Streptomyces thermoviolaceus is used industrially as a granaticin producer. Granaticin is polyketide-derived antibiotic as the secondary metabolite synthesized by Streptomyces thermoviolaceus. The molecular formula of granaticin is C20H20O10. It was isolated from Streptomyces olivaceus firstly and then detected in a few other actinomycetes (Snipes, Chang, and Floss, 1979). Granaticin can against Gram-positive bacteria while has few or no effect to Gram-negative, fungi, yeast or mycobacteria (Snipes, Chang, and Floss, 1979). The significance of the granaticin in industry is that it can be as protection to against competing of soil bacteria. It also was found can against the cancer cell such as human oral epidermoid cell (Heinstein,

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1982). Therefore, granaticin is interested in industry. The previous research shown that the production of granaticin is pH sensitive and related to the influence of carbon sources (James and Edwards, 1989). In the acidic conditions, the color of granaticin is red. Moreover, the color is blue when granaticin in alkaline environment. It convenient for observe the production circumstance of granaticin. In addition, a number of carbon sources such as glucose, proline and glutamate may affect the production of the granaticin (Hasani, Kariminik, and Issazadeh, 2014). Some studies found that granaticin grow in different temperatures. Edwards (1987, cited in Philip, 1991) refers Streptomyces thermoviolaceus grows between 25°C to 57°C however the production will be thermolabiled at temperatures beyond of 50°C. Therefore, In this experiment we apply the pH-limited continuous cultures to indicate the effects of cultural conditions include carbon source and temperature. In order to determine the optimal conditions, we assumed that glutamate is best carbon source and 45°C is optimal temperature. Therefore, the hypothesis was established which is Streptomyces thermoviolaceus can produce the maximum granaticin in defined medium which using the glutamate as the sole carbon source at 45°C.

2. Materials and Methods

2.1. Organism and Cultural Conditions

Streptomyces thermoviolaceus was grown overnight at a temperature range between 30°C and 50°C by demonstrator in incubator before the experiment. Then defined mediums were prepared. The each defined medium was contained (g L^{-1}): MOPS, 5.23; $(NH_4)_2SO_4$, 2; $MgSO_4.7H_2O$, 1; K_2HPO_4, 1 ; $CaCl_2.2H_2O$, 0.05 and 1 mL L^{-1} of a trace element solution that contained 1 g L^{-1} of each of following: $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$. The whole defined medium solution of 945 mL was separated and added into 27 labeled conical flasks for each of 35 mL. Then three different carbon sources which are glucose, proline and glutamate of each 10 mL solution were added into the labeled conical flasks. Finally the broth culture of 5 mL contain Streptomyces thermoviolaceus were added into 27 flasks and cultured at three different temperature which are 40°C, 45°C and 50°C for 48 hours with shaking at 13 rpm in incubator.

2.2. Measurement of biomass:

Each culture samples (10 ml) were harvested and placed in a pre-dried, pre-weighed container and dried to constant mass at in 50°C an oven. Then the biomass of each sample was measured on electronic balance. The data was recorded and analyzed.

2.3. Quantification of granaticin:

Each culture samples (2 ml) were harvested and pipetted into tube and centrifuged for 1min at 13000 rpm. Then samples were pipetted into bijoux and adjust pH into 11. Finally granaticin absorbance was measured by recording the optical density (OD) in the spectrophotometer at 602nm. The data was recorded and analyzed.

The optimal culture sample were cultured in shaking water bath at 45℃. Every hour the sample was harvested and pipetted into tube and centrifuged for 1 min at 13000 rpm. Test the optical density (OD) per hour. The data was recorded and analyzed.

3. Results

This experiment is going to determine the optimal carbon sources and temperature for best yields of granaticin. Therefore, the first step is identifying the optimum of the growth of the Streptomyces thermoviolaceus. Growth was determined by measuring the dry weight of biomass. It is intuitionistic state the effects of different conditions to the bacteria growth. The

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figure 1 shows that Streptomyces thermoviolaceus was cultured in defined medium containing different carbon sources at different tempertures for 48 hours. 10mL of suspension for each sample was collected and dried. Then the biomass was weighted. From the figure 1, it clearly shows that glutamate is best growth carbon source. The proline is the worst growth carbon source.

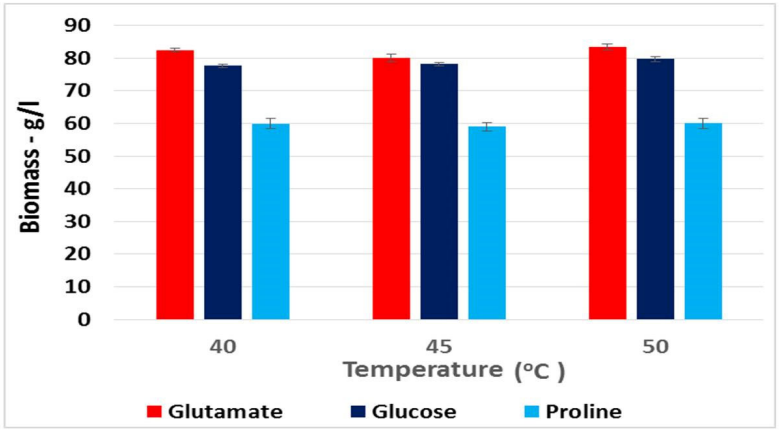

Figure 1. Effects of temperature and carbon sources on yields of biomass (g/l). X-axis is the temperature used for incubation. Y-axis is the biomass. Samples were set up in duplicate

The measuring of yields of granaticin is significant to demonstrate the optimal condition. The figure 2 shows the absorbance of the granaticin with culturing on three carbon sources (glucose, glutamate and proline) at three temperatures ((40°C, 45°C and 50°C) for 48 hours. Yields were determined by measuring absorbance at 602nm at pH 11. The figure provided that Streptomyces thermoviolaceus have lowest yields with culturing on glucose and the second yields with culturing on proline. Meanwhile, it is clearly shown that Streptomyces thermoviolaceus acquired the highest yields which cultured on glutamate. By comparing three temperatures, the yields of 45°C are slightly higher than the yields of 40°C. The yields of 50°C is minimum.

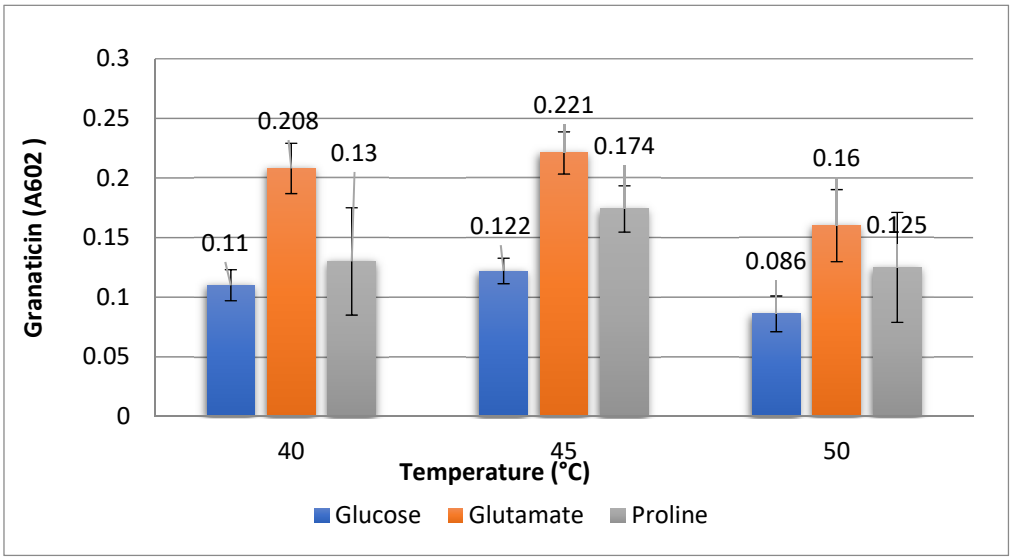

Figure 2. Effects of carbon sources and temperature on granaticin (g/l) yields. The absorbance was then read at 602nm (pH=11). X-axis is the temperature used for incubation. Y-axis is the absorbance of granaticin. Samples were set up in duplicate

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For the further understand of the production of granaticin yields. The Streptomyces thermoviolaceus grown at 45°C which using the glutamate as carbon source. Then the 2 mL of culture samples was collected hourly for measurment of granaticin. The figure 3 has shown that the producing is almost stationary from 0 to 1 hour and the granaticin was produced after the 1 hour. It also revealed the producing rate is higher after the 4 hours.

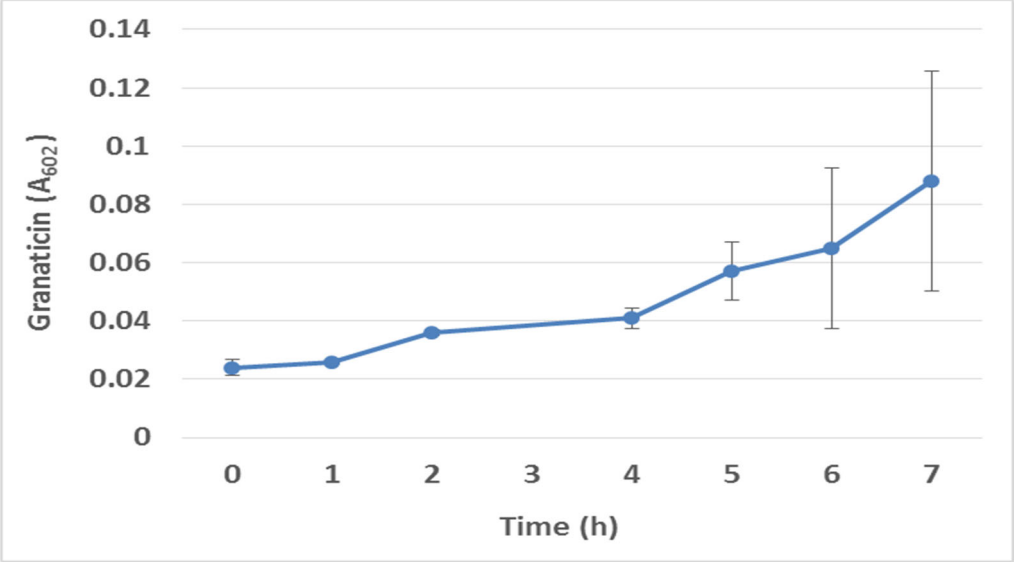

Figure 3. The fermentation for S. theromoviolaceus grown at 45°C in a defined medium using glutamate as a sole carbon source. 2ml of culture samples was removed hourly for quantification of granaticin. X-axis is the temperature used for incubation. Y-axis is the absorbance of granaticin at 602nm. Samples were set up in triplicate.

4. Discussion

Streptomyces thermoviolaceus is useful model for studying the second metabolism. It can grow promptly with suitable carbon source and produce the antibiotic easy to quantified and colorful. In addition, there are many factors may affect the production of Streptomyces thermoviolaceus such as carbon source, pH, oxygen and temperature (Hasani, Kariminik, and Issazadeh, 2014). This experiment implemented on effects of different carbon sources and temperature on granaticin yields. The objective of this experiment is to determine the optimal temperature and best carbon sources for granaticin production. From the results, the hypothesis is demonstrated correct. Glutamate was the best carbon source at 45 °C for secondary metabolism whilst glucose did not support secondary metabolism regardless of temperature. Meanwhile, the highest biomass was obtained at 50°C and lowest at 45°C.

The Figure 1 is proved that glutamate may result in higher growth of granaticin than glucose and proline. The surprised thing is that cultural sample using glucose has the lowest biomass. In a general way, glucose as the most common matter offering the energy usually can act the main carbon source for microbes. Lounes (1996, cited in Hasani, 2014) stated the reason that glucose can decrease the production of antibiotic because of the enzymes is suppressed. It may affect the antibiotic biosynthesis and growth rate on antibiotic biosynthesis. Hence, glucose is not suitable as the carbon source for the second metabolism of Streptomyces thermoviolaceus.

From the Figure 1, it is noticeable that 50°C is highest biomass of the Streptomyces thermoviolaceus and lowest at 45°C. However, the Figure 2 is shown that the maximum granaticin production occurs at 45°C. Thus it can be seen the optimal growth temperature is disparate to the maximum antibiotics production temperature. In this way, it should be discuss the reason cause that. Hasani (2014) referred that the growth of Streptomyces thermoviolaceus

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in exponential phase which implies there is an optimal growth rate gives rise to more antibiotic production. Therefore, here exists the relationship between first metabolite and second metabolite. James (1989) provided the evidence that although the production rate of the secondary metabolite is fastest at 37°C, the highest yield is seen at 45°C. He also mentioned the preference of producing biomass at 37°C. The most interesting about the granaticin producing is that there is a preference for granaticin but biomass at the intermediate temperature (45°C). When at the two extremes of temperature (30°C and 50°C), the preference for the biomass. Therefore, it can conjecture that there is inverse relationship connecting the biomass and antibiotic over the temperature range 30°C to 50°C.It means that the volumes of the biomass and antibiotic are suppressed by each other. There is the dynamic balance between them. Meanwhile, the growth of the biomass follows exponential phase. At the start of the incubation, the biomass will form rapidly until the substrate exhaustion (James and Edwards, 1989). Then it turns into the stationary phase and the second metabolite start to produce. Another interesting finding is that granaticin can be detected in culture samples before the exhausting of the glutamate. From the Figure 3, it can be seen that there is slightly increasing between zero time to 1 hour. Then the production rate is going to increase fast with time. It proves that second metabolism initiates before the cells stopped growing. Demain (1979, cited in James, 1989) also mentioned that trophophase and idiophase are separated in theoretically but can overlap in reality.

There are some factors may limiting the maximum of the growth. In lab experiment, the bacteria will not only response the substrate you given, but also affect with others nutrient. Inorganic and simple nitrogen sources may decrease antibiotic production (Hasani, Kariminik, and Issazadeh, 2014). Meanwhile, some studies shown that Streptomyces thermoviolaceus is a kind of thermosensitive bacterium has the respiratory chain. The respiratory chain will lose the NADH oxidase activity significantly above 40°C (James and Edwards, 1989). There are also some limitations in this experiment. For example, the sample size is too small to being persuasive. Time duration is few to record the change of the growth rate. They are all can be improved in future.

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