ORIGINAL ARTICLE

Growth and Penicillin Activities Resulted by *Penicillium chrysogenum* in Tomato (*Solanum Lycopersicum* L.) Juice

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ABSTRACT

Introduction: Penicillin is an antibiotic that has been mostly found and used in daily life. Penicillin is produced by the carbohydrate fermentation of Penicillium chrysogenum grown on the medium of nutrient agar or broth. In addition, P. chrysogenum can also be grown over a natural medium such as the enriched tomato juice. The tomato juice serves as the source of carbon, protein, and nutrition that the P. chrysogenum needs in performing the fermentation process. Regarding the statement, the growth of P. chrysogenum over the tomato juice as the medium can be studied through the biomass parameter, reducing the sugar through the Nelson-Somogyi method, and the penicillin activities resulted by the obstacle zone test. **Methods:** The tools that had been used in the study were test tube, LAF, oven, Petri dish, autoclave, spectrophotometer, vortex, pH meter microwave, incubator shaker, and water bath. **Results:** Then, based on the results of the study that has been conducted, it is found that the improvement of the biomass and the presence of the reduced sugar in the medium have shown the growth phase of P. chrysogenum. **Conclusion:** The penicillin growth and activities found in *Penicillium chrysogenum* grown over the tomato juice as the medium has indicated the presence of the growth phase.

Keywords: Biomass, Tomato Juice, Nelson-Somogyi, Penicillium chrysogenum, Obstacle Zone

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INTRODUCTION

Penicillium chrysogenum is one of the important microorganisms in the domain of industry because this microorganism is related to the production of commercial antibiotics known as penicillin (1). *P. chrysogenum* is mesophilic and this microorganism can grow at the minimum degree of 4°C, at the optimum degree of 23°C, and at the maximum degree of 37°C. The optimum of acidity (pH) for the growth of P*enicillium chrysogenum* ranges from 4 to 6 (2).

The production of penicillin as a secondary metabolite product depends on the optimum growth environment, namely temperature, medium/nutrient composition, aeration, and agitation (2). In addition, penicillin is also able to obstruct the growth of positive gram bacterial by interrupting the synthesis process and the cell wall (3). In the fermentation process, one of the classical methods that can be adopted in determining the quantitative amount of reducing sugar is the Nelson-Somogyi Method (4). There are phases of *P. chrysogenum* growth in relation to the biomass. The first phase is known as the lag phase, which lasts from 0 to 7 days. Then, the second phase is known as the log phase; in this phase, the biomass is doubled per time unit and this phase lasts from 8 to 15 days (5).

Medium serves to grow the cell outside its natural habitat. In the context of the study, the medium that will be used is the natural one namely tomato juice (*Solanum lycopersicum* L.) (6). Tomato contains alkaloids, folic acid, malic acid, citric acid, flavonoids, fat protein, sugar (glucose and fructose), adenine, trigonelline, chlorine, tomatin, mineral, and vitamin (B1, B2, B6, C, E, and lycopene) (7). As a result, tomato juice can be used as the source of carbon, protein, and nutrition in order to meet the needs of the microbial that has been planted (8).

In relation to the growth phase, the obstacle zone refers to the zone that has been formed because the bacterial does not grow due to the influence of the external factors (9). The resistance capacity of a substance to bacterial is defined by the diameter of the crystal-clear zone that has been formed (10). In order

to identify the resistance toward numerous antibiotics or to define the level of bacterial vulnerability toward the antibacterial substance, an obstacle zone test should be performed (11). The bigger the diameter is, the more obstructed the growth of the bacterial will (12).

MATERIALS AND METHODS

The tools that had been used in the study were test tube, LAF, oven, Petri dish, autoclave, spectrophotometer, vortex, pH meter microwave, incubator shaker, and water bath. Then, the materials that had been used were *Penicillium chrysogenum* starter, tomato juice, distilled water, NA powder, nelson (A+B) reagent, commercial penicillin, and Blanco solution.

Biomass Measurement

The Petri dish and the filter paper were weighed. Then, culture was taken and was poured into the Petri dish and the filter paper. Next, the Petri dish, the filter paper, and the culture were put into the oven for two days. Afterward, the Petri dish was weighed and the weight was recorded as the final weight.

Nelson-Somogyi Test

After the dilution had been complete, both of the sampled solution and the Blanco solution were ready and were measured in terms of absorbance by using the spectrophotometer with a wavelength of 540 mm. The formula for the Nelson-Somogyi test:

$$a = \frac{(\sum y)(\sum x^2) - (\sum x)(\sum xy)}{(\sum x)(\sum x^2) - (\sum x)^2}$$

$$b = \frac{(\sum x)(\sum xy) - (\sum x)(\sum y)}{(\sum x)(\sum x^2) - (\sum x)^2}$$

$$y = a + bx$$

Obstacle Zone Test

The culture of Bacillus subtilis and Escherichia coli was taken in a different medium. The size and the percentage of the obstacle zone were calculated using the two formulas below:

$$\text{Size of obstacle zone} = \varpi \left(\left(\frac{D1}{2}\right)^2 - \left(\frac{D2}{2}\right)^2 \right)$$

$$\% \text{ obstacle zone} = \frac{\textit{Size of Obstacle Zone (z)}}{\textit{Size of Petri Dish}} \; Ч100\%$$

RESULTS

Based on the results of the observation that had been performed for seven days, the following graphic was attained (Figure 1).

In addition to weighing the biomass, the observation on the growth of *P. Chrysogenum* was also conducted by measuring the pH value of the medium (Figure 2).

Then, the growth pattern of *P. Chrysogenum* was identified by measuring the reducing sugar through the implementation of the Nelson-Somogyi Method (Figure 3).

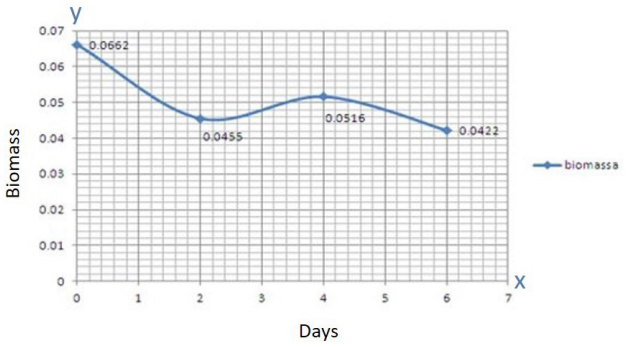

Figure. 1 : The Biomass Graphic of *P.chrysogenum.*

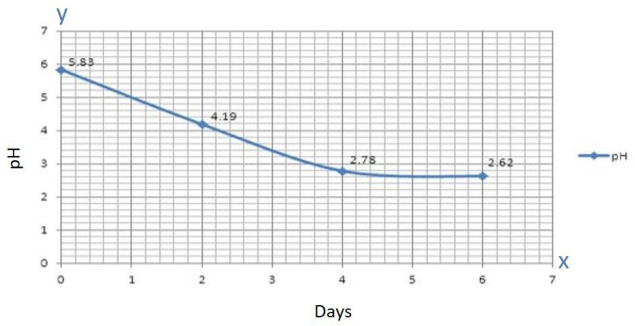

Figure. 2 : The pH Graphic of *P. Chrysogenum.*

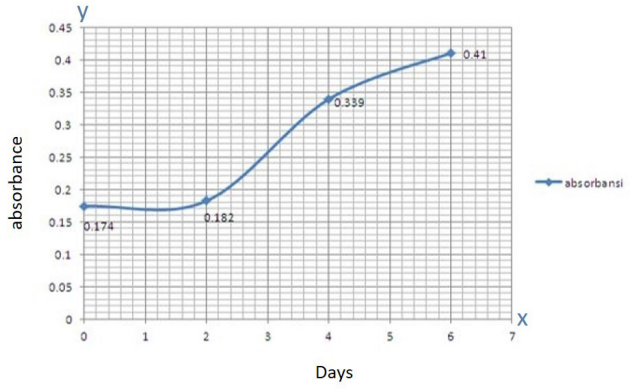

Figure. 3 : The Graphic of Reducing Sugar.

With these characteristics, the obstacle zone could be performed. The obstacle zone test was performed by using the positive gram bacteria *Bacillus subtilis* and the negative gram bacterial *Escherichia coli*. The results of the obstacle zone test were described by the two figures below (Figure 4).

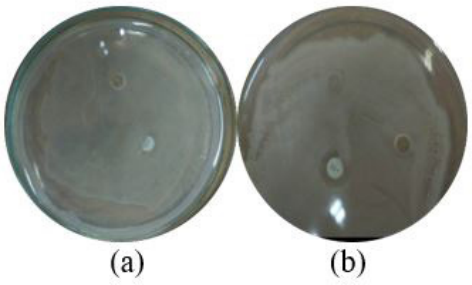

Figure. 4 : The Obstacle Zone of (a) *E. coli* and (b) *B. subtilis*.

DISCUSSION

P. chrysogenum as filamentous fungi will experience mycelium growth at the beginning of the incubation and, consequently, the biomass will increase. When *P. Chrysogenum* starts to synthesize the penicillium, the mycelium growth will stop and therefore biomass change will not occur. Both aspects are unseen in the observation results portrayed by Fig.2. Then, the second day of incubation belongs to the lag phase. In this phase, generally, the mycelium growth does not change but the observation results indicate biomass decrease. The biomass decrease can be caused by the death of the cell in an inappropriate environment.

Then, the graphic in Fig.3 shows that there has been an absorbance increase. The absorbance increase indicates the decrease in the fermentation activities by *P. chrysogenum*. Such decreasing activities can be caused by the inhibiting factors in the tomato juice as the medium or the mismatch between the fungi and the nutrition in the medium. The consequence of such decreasing activities is that fungi growth has stopped.

Eventually, from the graphic in Fig.4 it is found that the obstacle zone has not bee formed in the well holes of both the *E. coli* medium and the *B. subtilis* medium. On the contrary, the obstacle zone in the disc of commercial Penicillium for both of the *E. coli* medium and the *B. subtilis* medium has been formed.

CONCLUSION

The penicillin growth and activities found in *Penicillium chrysogenum* grown over the tomato juice as the medium has indicated the presence of the growth phase. However, the penicillin activities that have been resulted are not effective in form the obstacle zone on the bacterial that has been defined.

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