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The addition of various carbon sources on growing media to increase the siderophore level of fluorescent pseudomonad bacteria

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Abstract. Fluorescent pseudomonad is a member of the rhizobacterial groups which has the potential to be developed as an agent for inducing plant resistance. Several species of fluorescent pseudomonad are capable of producing siderophore. Siderophore is an organic antimicrobial compound that plays a role in biological control of plant diseases. The aim of the study was to determine the best carbon source added to the growing media to increase the levels of siderophore from the fluorescent pseudomonad PfCas3 and PflAHp2 isolates. This research is an experimental study that use a completely randomized design with 4 treatments, which is the addition of carbon sources of glucose, fructose, glycerol on growing media, and King's B medium (control), and 3 replications. Detection of siderophore was measured with a spectrophotometer at a 410 nm wavelength (λ). The data obtained were analyzed using ANOVA and DNMRT continued testing at a significant level of 5%. The results showed that the best carbon source added to the fluorescent pseudomonad PfCas3 isolate growing medium was glucose, with the siderophore level produced 1,574 at $\lambda = 410$ nm. Glucose added to the growing medium can also increase the levels of siderophore from the fluorescent pseudomonad PflAHp2 isolate, which is 1,464 at $\lambda = 410$ nm.

1. Introduction

Fluorescent pseudomonad is a group of rhizobacteria which has the potential to be developed as an agent for inducing plant resistance. This bacterial group can be isolated from the root area of the plant by not considering its function in the root area. Chatri[1] stated that several types of rhizobacteria can suppress plant diseases through resistance induction responses and can also increase plant growth. Deshwal and Kumar [2] reported that several species of fluorescent pseudomonads were able to produce siderophore, hydrogen cyanide antimicrobials, IAA, and phosphate solvent compounds, and also showed plant growth booster activity.

Siderophore is an antimicrobial organic compound that acts as biological control of plant diseases that have very high iron affinity, dissolve in water and rapidly diffuse. The presence of siderophore compounds enables fluorescent pseudomonads to dissolve phosphorus that needed by plants, so that plant growth is better and resistant to diseases [3]. Advinda[4] reported that fluorescent pseudomonads PfPj1, PfPj2, PfCas3, PflAHp2, PfPb2, PfPb3 and PfPm1 isolates were able to subdue the growth of Blood Disease Bacteria (BDB), also escalate the growing of banana plants.



Microbial growth can occur due to the presence of nutrients as an energy source. Nutrients are organic and inorganic substances in the form of solutions that can cross the cytoplasmic membrane. Nutrients are needed by microbes as carbon sources, nitrogen sources, certain inorganic ions, vitamins, and water [5]. Advinda, *et al.*, [6] reported that the growth and multiplication of fluorescent pseudomonads PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2 isolates required a growing media. The chemical composition or mineral salts in the growing media might affect the production of antimicrobials (such as hydrogen cyanide) of these bacteria.

Carbon sources are needed by microbes for their growth and development. According to Hogg [7], carbon sources are also very influential on the production of antimicrobial compounds, transcription, promotion of biosynthesis, nutrient availability and pH in addition to maintaining cell integrity, as well as enzyme and protein catalysts. Addy [8] reported that carbon sources of fructose, glucose, glycerol, and mannitol have different roles in stimulating the production of fluorescent pseudomonad antimicrobial compounds. This is indicated by the difference in the formation of inhibitory zones by fluorescent pseudomonads against *Erwinia carotovora* subsp. *carotovora* in the test medium.

Streptomyces spp. isolates of LSW05, LBR02, and SSW02 were able to grow on *Yeast Malt Agar* medium, while PS4-16 grew well in *Oatmeal Agar* medium. This growth difference shows that the ability of isolates in utilizing carbon sources is also different [9]. Coconut water can be used as a fluorescent pseudomonad growing medium. The highest bacterial count was found after 6 weeks of incubation (msi), and a decrease in the number of bacteria occurred on 8 msi [10]. Zhou, *et al.*, [11] reported that the highest biomass (bacterial count) of the *Pseudomonas brassicacearum* J12 strain can be produced if there is glucose as a carbon source in the growing media. This study aims to determine the best carbon source to be added to the growing media in order to increase the siderophore levels from fluorescent pseudomonads PfCas3 and PfLAHp2 isolates.

2. Material and methods

Fluorescent pseudomonad isolates used were PfCas3 and PfLAHp2 (collection Advinda 2007 and 2017). This research was an experimental study which used a completely randomized design with 4 treatments which is the addition of carbon sources: glucose, fructose, glycerol on the growing media, and King's B media (control), and 3 replications. Detection of siderophore was measured using a spectrophotometer at a 410 nm wavelength (λ).

2.1. Rejuvenation and propagation of fluorescent pseudomonad

Fluorescent pseudomonad PfCas3 dan PfLAHp2 isolates were revived on solid King's B media, and incubated for 2x24 hours. The inoculum propagation was accomplished by taking a pure culture ose in petri, then bred in 25 mL King's B liquid media which was shake for 24 hours.

2.2. Preparation of the growing media of fluorescent pseudomonad

The making of King's B + glucose (with 1,5% concentration) media was conducted by weighing 5g of peptone protease, 0,375 g of K_2HPO_4 , 0,375 g of $MgSO_4 \cdot 7H_2O$, and 3,75 g of glucose. Then the mixture of the ingredients was inserted into beaker glass, and subsequently distilled water was added to a volume of 250 mL. The media sterilization was done using an autoclave at 121 °C and 15 psi of pressure for 15 minutes. The preparation of the other media (addition of fructose 3,75 g, and glycerol 3,75 mL) was accomplished in the same manner.

The production of King's B media (control) was accomplished by weighing 5g of peptone protease, 0,375 g of K_2HPO_4 , 0,375 g of $MgSO_4 \cdot 7H_2O$, and 2,5 mL of glycerol. Then the mixture of the ingredients was inserted into beaker glass, and distilled water was added to a volume of 250 mL. The media sterilization was done using an autoclave at 121 °C and 15 psi of pressure for 15 minutes.

2.3. The siderophore capability test media creation

The siderophore production media consist of 20 g of sucrose, 2 g of L-asparagine, 1 g of K₂HPO₄, and 0.5 g of MgSO₄ dissolved into aquades up to a volume of 1,000 mL. The media sterilization was done using an autoclave at 121 ° C and 15 psi of pressure for 15 minutes.

2.4. Cultivation of fluorescent pseudomonad PfCas3 and PflAHp2 isolates

The existing fluorescent pseudomonad PfCas3 and PflAHp2 isolates were grown on each growing prepared medium. This was accomplished by taking 1 mL of 3x10⁸ cells /mL (scale 1 Mc Farland's) of the fluorescent pseudomonad suspension, then transferring it into 25 mL of treatment media which was shake for 24 hours at room temperature.

2.5 Siderophore production

Fluorescent pseudomonad PfCas3 and PflAHp2 isolates that have grown on the treatment media were examined for their ability to produce siderophore. This is done by taking 1 mL of suspension, then transferring it to 25 mL test medium for siderophore and shaken for 24 hours at room temperature. The resulting suspension was taken as much as 10 mL which then centrifuged at 3,000 rpm for 10 minutes. The supernatant was taken using micropipette. Detection of siderophore production was carried out by adding 1 mL FeCl 0.01 M into 3 mL supernatant, while the control used was supernatant without the addition of FeCl. Detection of siderophore was measured with a spectrophotometer at of 410 nm wavelength [12].

3. Results

The addition of various carbon sources in the fluorescent pseudomonad PfCas3 and PflAHp2 growing media has produced different levels of siderophore. The carbon sources used are glucose, fructose and glycerol. Siderophore levels were determined using a spectrophotometer at a 410 nm wavelength, and the resulting amount was a number of Optical Density (OD). The highest siderophore production was 1,574 (OD 410 nm), produced by the fluorescent pseudomonad PfCas3 grown on glucose media. Glucose media can also increase the production of siderophore from fluorescent pseudomonad PflAHp2, which is 1,354 (OD 410 nm). While the fluorescent pseudomonad PflAHp2 produced the lowest number of siderophore in fructose media, i.e. 0.435 (OD 410 nm). Siderophore production from fluorescent pseudomonad PfCas3 and PflAHp2 grown in various carbon sources can be seen in Table 1.

Table 1. Siderophore production by fluorescent pseudomonad PfCas3 and PflAHp2 in the growing media which added several carbon sources.

Treatment	Siderophore (OD 410 nm)
PfCas3+glucose	1,574 a
PflAHp2+ glucose	1,354 b
PflAHp2+KB (control)	1,027 c
PflAHp2+glyserol	0,928 c
PfCas3+ glyserol	0,567 d
PfCas3+ KB (control)	0,533 d
PfCas3+fructose	0,528 d
PflAHp2+ fructose	0,435 d

4. Discussion and Conclusion

The role of siderophore produced by fluorescent pseudomonad as a bio-control agent for plant diseases has been widely reported. Ramyasmruthi *et al.*, [13] reported that fluorescent pseudomonads isolate R was a bio-control agent that produced siderophore, so it was able to inhibit various plant pathogens such as *Collectotrichum gleosporioides*, *Alternaria brassicola*, *A. brassiceae*, *A. alternate*, *F. oxysporum*, *Rhizoctonia solani*, and *Phytophthora* with various levels of attack. According to Patel *et*

al., [14], the ability of siderophore produced by bio-control agent *Bacillus* spp in binding iron is a competitor to other microorganisms, so that in the agricultural system it can be used as a control for plant diseases, and improvement of plant growth.

Nutrient content in growing media of fluorescent pseudomonad can affect siderophore production [15]. Duffy and Defago [16] reported siderophore in the form of pyoluteorin and pyrrolnitrin produced by fluorescent pseudomonad CHA0 can be increased by adding glucose to the growing media. While Kumar [17] reported that bacterial isolates VITVK5 and VITVK6 isolated from the soil can produce siderophore. The impact of the three carbon sources (glucose, fructose, and sucrose) added to growing media of these two isolates showed that bacterial isolates VITVK5 produced the highest siderophore in sucrose media.

This study concluded that the addition of various carbon resources to fluorescent pseudomonad growing media can affect the production of siderophore. The fluorescent pseudomonad PfCas3 and PflAHp2 were each able to produce the most siderophore in the media which added a carbon source in the form of glucose. If fructose is used as a carbon source, it results in the lowest production of siderophore in isolates PflAHp2.

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