The Addition of Several Mineral Sources on Growing Media of Fluorescent Pseudomonad for the Biosynthesis of Hydrogen Cyanide

IOP Conf. Series: Materials Science and Engineering **335** (2018) 012016 doi:10.1088/1757-899X/335/1/012016

The Addition of Several Mineral Sources on Growing Media of Fluorescent Pseudomonad for the Biosynthesis of Hydrogen Cyanide

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Abstract. All Fluorescent pseudomonad is a group of rhyzobacteria which these days often utilized on plant disease control. The growing media is an absolute requirement which needs to be considered for the growth and cultivation of bacteria. The mineral source contained in growing media of bacteria may affect the production of hydrogen cyanide compound. The objectives of the research were to obtain the best source of minerals for biosynthesis of cyanide acid compounds by fluorescent pseudomonad isolates PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2. This research is a qualitative experimental research including observation of hydrogen cyanide compound produced after the growing media of fluorescent pseudomonad bacteria added with several mineral sources. The treatments were given: A = ZnSO4.7H2O 0.5 mM addition, B = CoCl2.6H2O 0.5 mM addition, and C = Fe2SO4.7H2O 0.5 mM addition. From the result of the research, it was concluded that the addition of ZnSO4.7H2O mineral resources on the growing media of fluorescent pseudomonad isolate Cas and Cas3 produced the best hydrogen cyanide. Whereas addition of CoCl2.6H2O mineral source on the growing media showed poor hydrogen cyanide production for all fluorescent pseudomonad isolates

1. Introduction

Fluorescent pseudomonad is a biocontrol agent that can be isolated from the surface area of plant roots. This group of bacteria is non-pathogenic, which can suppress pathogenic pathogens through rhizosphere colonization, producing siderophores, antimicrobial hydrogen cyanide(HCN), IAA, phosphate solvent compounds, and induced systemic resistance. Paul and Sarma [1] reported fluorescent pseudomonad can suppress root rot disease in black pepper plants caused by Phytophthora capsici. According to Advinda [2], fluorescent pseudomonad isolates PfPj1 can inhibit the growth of Ralstonia solanacearum bacteria, and also able to increase the growth of banana plants. Furthermore, Advinda [3] reported fluorescent pseudomonad isolates PfPj1, PfPj2, PfPj3, PfPb1, PfPb2, PfPb3 and PfPm1 can suppress the development of Blood Disease Bacteria (BDB) in banana seedlings. According to Doktycz, et al., [4] Pseudomonas fluorescens GM30 strains can be used for Populus delloides plant defense against pathogens, as well as increasing their growth.

The growth and propagation of biocontrol agents require growing media. The chemical composition contained in the growing media may affect the growth of the biocontrol agent. Antimicrobial/antibiotic production by biocontrol agents is influenced by chemical compositions or mineral salts that constitute a growing media. The production of antibiotics from Streptomyces sp.

MS-266 Dm4 is affected by various concentrations of mineral salts provided such as: K2HPO4, MgSO4.7H2O, and KCl. The maximum antibiotic concentration was produced at K2HPO4 concentrations of 0.2 g/100 mL, MgSO4.7H2O 0.15 g/100 mL, and KCl 0.05 g/100 mL [5]. Addition of Zn2+, Cu2+, and NH4Mo2+ on growing media of P. fluorescens CHAO can produce 2,4-diacetyl phloroglucinol (DAPG) antimicrobial compounds [6]. This study explored the biosynthesis of hydrogen cyanide by fluorescent pseudomonad isolates PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2 that is grown on growing media with the addition of different mineral sources.

2. Material and methods

This research is a qualitative experiment research including observation of hydrogen cyanide compounds produced after the growing medium from pseudomonad fluorescent were added with several mineral sources. Fluorescent pseudomonad used are isolates PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2. Mineral salts added in King's B growing medium are ZnSO4.7H2O 0.5 mM, CoCl2.6H2O 0.5 mM, and Fe2SO4.7H2O 0.5 mM.

2.1. Rejuvenation and propagation of fluorescent pseudomonad

Fluorescent pseudomonad isolates PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2 were rejuvenated on solid King's B medium, and incubated for 2x24 hours. The inoculum propagation was carried out by taking a pure culture ose in petri, then bred in 25 mL King's B liquid medium, and is shake for 24 hours.

2.2. Preparation of growing medium of fluorescent pseudomonad

The making of King's B + ZnSO4.7H2O 0.5 mM media was done by weighing pepton protease by 20 g, 1.5 g of K2HPO4, 1.5 g of MgSO4.7H2O, 10 mL of glycerol, 10 mL of ZnSO4.7H2O 0,5 mM, and 18 g of gelatin. Then the mixture of the ingredients is inserted into beaker glass, and distilled water added to a volume of 1,000 mL. Sterilization of the media was carried out using an autoclave at 121 ° C and 15 psi of pressure for 15 min. The preparation of 2 other media (addition of CoCl2.6H2O 0.5 mM, and Fe2SO4.7H2O) was carried out in the same manner..

2.3. The making of hydrogen cyanide production trial media

The production media of hydrogen cyanide consist of 4.2 g of glycine and 10 g of TSA which is dissolved into aquadest to 1 Liter volume. Then sterilized in autoclave at 121^{o} C and pressure of 15 psi for 15 minutes. The indicator of hydrogen cyanide compound production is a solution consisting of 2 g of picric acid and 8 g of sodium carbonate dissolved into aquadest to 200 mL volume [7].

2.4. The cultivation of isolates PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2

The fluorescent pseudomonad isolates PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2 were grown on each prepared media by taking 1 mL of pseudomonad fluorescent suspension (1 Mc Farland's scale). Furthermore, the suspension is inserted into a sterile petri dish, and poured the media according to the treatment. Bacteria cultures were incubated at room temperature for 2x24 hours. The ability of each isolate to grow on each treatment medium given was observed.

2.5. The propagation of isolates PfPj1, PfPb1, PfPj2, Kd7, Cas, Cas3, and LAHp2

Propagation of isolates was carried out for the trial of hydrogen cyanide production. Isolates that have been grown in each mineral addition to the media treatment, are propagated using the same media according to the treatment but with no addition of gelatin (liquid media). Inoculum propagation was performed by taking an ose culture in petri, then bred in 25 mL of liquid medium, and was shake for 24 hours.

2.6. The production of hydrogen cyanide

Isolates that have grown were implanted in glycine media in a petri dish with a cast method. Pieces of filter paper that have been spilled 1 mL hydrogen cyanide detector solution were embedded on the lid of petri dish. Bacteria cultures were incubated at room temperature for 2x24 hours. A change of color of the filter paper from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (++) or strong (+++) reaction respectively.

3. Results

3.1. The living ability of the fluorescent pseudomonad in a growing media with addition of several minerals

The best living ability in King's B + ZnSO4.7H2O growing media is found in 4 fluorescent pseudomonad isolates, i.e. isolates PfPj1, Kd7, Cas, and Cas3. The four isolates had the highest bacterial count compared to the other three isolates. The PfPj1 isolate had the best living ability in the growing media of King's B + CoCl2.6H2O, but the isolates of PfPb1 were not able to grow. In the growing media of King's B + Fe2SO4.7H2O, Cas3 isolates are not able to grow. The other six pseudomonas isolates were able to grow on King's B + Fe2SO4.7H2O (Table 1). The growing media ZnSO4.7H2O, CoCl2.6H2O, and Fe2SO4.7H2O used can be seen in Figure 1.

Table 1. The number of bacteria that grow on several different minerals added media (CFU / mL).

No	Isolate	Media type		
		KB+Zn	KB+Co	KB+Fe
1	PfPj1	30.10^{11}	202.10^{10}	$101.\ 10^{10}$
2	PfPb1	35.10^{10}	-	$160.\ 10^{10}$
3	PfPj2	202.10^{10}	110.10^{9}	$176.\ 10^{10}$
4	Kd_7	30.10^{11}	3.10^{9}	$122.\ 10^{10}$
5	Cas	30.10^{11}	50.10^{9}	$176.\ 10^{10}$
6	Cas3	30.10^{11}	3.10^{9}	-
7	LAHp2	225.10^{10}	6.10^{9}	$16.\ 10^{10}$

(a) (b) (c)

Figure 1. (a) ZnSO4.7H2O, (b) CoCl2.6H2O, and (c) Fe2SO4.7H2O media

3.2. The production of hydrogen cyanide

Study analysis of hydrogen cyanide production showed the addition of ZnSO4.7H2O mineral source is best in producing hydrogen cyanide by fluorescent pseudomonad isolate Cas and Cas3. In the CoCl2.6H2O-added growth media, all isolates showed poor hydrogen cyanide production from none to weak. Isolate PfPb1, PfPj2, and LAHp2 did not produce hydrogen cyanide on growth media with addition of CoCl2.6H2O and Fe2SO4.7H2O (Table 2.). The production of hydrogen cyanide by fluorescent pseudomonad will be seen by the differences in color seen on the filter paper. The color of the yellow filter paper indicates that no hydrogen cyanide is produced, the light brown is produced by hydrogen cyanide but the reaction is weak, the brown color generated by moderate reaction hydrogen cyanide, while the strong reaction exhibits a reddish brown color (Figure 2).

Figure 2. Hydrogen cyanide production by fluorescent pseudomonad. (A) ZnSO4.7H2O growing media, (B) CoCl2.6H2O growing media, and (C) Fe2SO4.7H2O growing media

Table 2. The production of hydrogen cyanide from pseudomonad fluorescent.

No	Isolate	Media type		
		KB+Zn	KB+Co	KB+Fe
1	PfPj1	++	+	++
2	PfPb1	+	-	-
3	PfPj2	++	+	++
4	Kd_7	+++	+	++
5	Cas	+++	+	++
6	Cas3	+++	+	++
7	LAHp2	+	-	-

- None
+ Weak
++ Moderate
+++ Strong

4. Discussion and Conclusion

The role of hydrogen cyanide in suppressing various plant diseases has been reported by several researchers. Hydrogen cyanide and ammonia synthesis were observed in all the bacterial isolates. Kumar et al., [8] reported that some isolated pseudomonad produce hydrogen cyanide as the primary mechanism of biocontrol. Prasad et al., [9] states that hydrogen cyanide is not only produced by biocontrol agents of Pseudomonas fluorescens, but also by Bacillus subtilis, Trichoderma viride, and T. harzianum. Screening of six isolates of P. fluorescens found only one isolate producing strong hydrogen cyanide, while the other five were moderate. According to Ramette et al., [10] in addition to increasing plant growth, Pseudomonas can release hydrogen cyanide to the rhizosphere of tobacco, thereby suppressing Thieviopsis basicola, causal agent of black root rot of tobacco.

The addition of Zn and Fe to the growing media, can enhance the ability of bacteria as a biocontrol agent. Addition of ZnSO4 to soil as a growing media of fluorescent pseudomonad isolates UTPF5 and MPFM1 could significantly increase the growth of bean crops, while for UTPF76 isolates there was a decrease in growth. Zn is an essential co-factor for changing the tryptophan amino acid into auxin (growth stimulant hormone) [12].

From the research, it was concluded that the addition of ZnSO4.7H2O mineral resources to the growing media of fluorescent pseudomonad isolate Cas and Cas3 produced the best hydrogen cyanide. While the addition of CoCl2.6H2O mineral source on growing media showed poor hydrogen cyanide production for all pseudomonad fluorescent isolates.

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