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Inoculating of *Pseudomonas putida* IFO 14796 on the early growth of corn seed

Jumadi¹, Saifuddin Sirajuddin², M.Natsir Djide³ and Anwar Mallongi⁴

¹Postgraduate Student of Public Health Department, Hasanuddin University, Makassar City, South Sulawesi, Indonesia

²Department of Nutrition, Faculty of Public Health, Hasanuddin University, Makassar City, South Sulawesi, Indonesia

³Departments of Pharmacology, Hasanuddin University, Makassar City, South Sulawesi, Indonesia

⁴Department of Environmental Health, Faculty of Public Health, Hasanuddin University, Makassar City, South Sulawesi, Indonesia

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ABSTRACT

Biofortification using plant growth promotion (PGP) bacteria for agronomic intervention is one of the most important strategies to enhance the growth and quality of the maize grain. This study aims to assess the ability of *Pseudomonas putida* IFO 14796 to stimulate the growth of corn plant. Randomized group design was carried out to assess the effect of *Pseudomonas putida* IFO 14796 on the early growth of seed corn with three levels of treatment and one of control and 3 replications respectively. The density of *P. putida* IFO 14796 in the treatment of 3:1 carrier was (5.1x10⁷ CFU g⁻¹), (1.37x10⁷ CFU g⁻¹), and 1:1 (0.37x10⁷ CFU g⁻¹) and control (uninoculated). The carrier and topsoil were sterilized in an autoclave at 121°C and pressure of 15 pounds for 1.2 h. Parameters of the root length, number of roots, number of leaves, and plant biomass were collected after 96 h. Data were analyzed by ANOVA and LSD. The effect of the *Pseudomonas putida* IFO 14796 on the primary root length of the corn plants was significantly different at p<0.05. The mean of the primary root length of 3:1 (19.53 cm plant⁻¹) was significantly different over the other treatments at p<0.05. *P. putida* IFO 14796 can stimulate the elongation of the root corn plant so it is potentially developed for improving the quality of the maize grain based on biofortification strategies for chronic diseases prevention. The current work is iron biofortification of corn seed through *P. putida* IFO 14796 intervention.

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Key words : Corn seed, *Pseudomonas putida* IFO 14796, Early growth, Plant growth promotion

Introduction

The plant growth promotion rhizosphere (PGPR) has been developed to stimulate the growth and production of the corn plant. The bacteria of PGP consisted of the species of *Pseudomonas stutzeri*, *Bacillus subtilis*, *Stenotrophomonas maltophilia*, *Bacillus amyloliquefaciens*, *Rhodopseudomonas palustris*, *Enterobacter ludwigii*, *Lactobacillus plantarum*,

Strobacterium citreum, and *Pantoea punctate* (Islam *et al.*, 2015; Sakpinim *et al.*, 2017). However, *Pseudomonas putida* was one of the most dominant species founded in root tissue of corn plant as endophytic (de Melo Pereira *et al.*, 2012), forming the root colonization in maize (Neal and Ton 2013), symbiotic interaction plant-bacteria (Vacheron *et al.*, 2018), and forming the root systems of agricultural crops in cereal (Marrero *et al.*, 2015).

*Corresponding author's email : undjumadi@gmail.com

¹Dayanu Ikhsanuddin University, Baubau-Southeast Sulawesi

Pseudomonas sp. isolated from corn rhizosphere is able to solubilize phosphate complexes into plant absorbable and utilizable forms (Li *et al.*, 2017) that plays an important role to stimulate the growth of roots, shoots, plant weight, number of leaves, number of seeds, iron contents of corn seed, protecting plants against the phytotoxicity (Han *et al.*, 2014; Lopes *et al.*, 2018). Besides that, *Pseudomonas putida* has the ability to produce Indole-3-Acetic Acid (IAA) and siderophores, gibberellins (GAs), cytokinins (CTKs), ethylene, extracellular enzymes, antibiotics and cyanide which gave a positive effect on plant growth (Iqbal Ahmad and Hayat, 2008; de Menezes *et al.*, 2012) and nitrogen-fixing ability to increase in nitrogen content, biomass accumulation and nitrogen fixation rates in major cereal crops (Fox *et al.*, 2016). The present research was carried out to assess the ability of *P. putida* IFO 14796 to stimulate the early growth of corn seeds.

Materials and Methods

Experiment Materials

Pseudomonas putida IFO 14796 (Osaka Japan collection) was accessed from The Laboratory of Food and Nutrition, University of Gajah Mada, Indonesia. The proliferation of *P. putida* IFO14796 was carried out in potato extract- sucrose broth (PESB) at 28°C (Martyniuk and Oron 2011). The carrier was made from a combination of green compost and rice bran in particle size <0.5 mm with the 3:1, 2:1, and 1:1 proportion and then stored in the closed package for sterilized in an autoclave at 121 °C, pressure 15 pounds for 1.2 h (FNCA 2006). By sterilized, the carrier was enriched *P. putida* IFO (7.83×10⁷CFU mL⁻¹) and then incubated at room temperature for three months.

Table 1. The effect of *Pseudomonas putida* IFO 14796 on the plant biomass, number of roots, length of primary root, and number of leaves of the corn plant

Treatment	Success (%)	Plant Biomass (g plant ⁻¹) ^{ns}	Number of Roots (NOR) (Unit plant ⁻¹) ^{ns}	Length of Roots (LOR) (cm plant ⁻¹)*	Number of Leaves (sheet plant ⁻¹)
Control	100	1.09	5.3	15.67	2
A (1:1)	100	0.99	5.3	17.17	2
B (2:1)	100	1.19	4.7	16.96	2
C (3:1)	100	1.22	4.7	19.53	2

* mean difference is significant at p<0.05;
- ns (non-significant) at p<0.05

Polybag containing a combination of top soil and sand obtained from agriculture land in particle size <1.5 mm were sterilized in an autoclave at 121°C and pressure 15 pounds for 1.2 h which was used in the present study. The corn obtained from the Cereals Research Center-Maros, South Sulawesi-Indonesia. Corn seeds sterilized in 70% ethanol for 5 minutes, and then were washed with distilled water three times repeated (Le Doyen and Kropf 2013). The population density of *P. putida* IFO 14769 in the treatment of 3:1 was 5.1×10⁷ CFU g⁻¹, 2:1 (1.37×10⁷ CFU g⁻¹) and 1:1 (0.37×10⁷ CFU g⁻¹).

Design Experiment

The experiment was carried out by randomized group design consisted of 3 treatment groups and 1 control group, and 3 replications respectively. By randomized, each polybag was grown one corn seed then placed in every group for 96 h cultivation. The plant biomass was measured manually by digital scale 100g x 0.1 G and the root length by a ruler in millimeter.

Statistical Analysis

Data were analyzed by ANOVA (Analysis of Variance) and Fisher's protected least significant difference (LSD) tested used the software package SPSS 15.0.

Result

The Early Growth of Corn Seed in The Sterilized Soil

The corn seeds in the experiment unit have been able to grow by up to 100%. Based on the analysis of variance (ANOVA) revealed that there was the effect of *P. putida* IFO 14796 on the primary root

length at $p < 0.05$. Meanwhile, the plant biomass ($1.22 \text{ g plant}^{-1}$) and the primary root length ($19.53 \text{ cm plant}^{-1}$) of the 3:1 were highest over the other treatments and control (Table 1).

During the early growth of corn seeds show the average of the primary root length in the treatment of C (3:1) was (19.53 cm) higher than A (17.17), B (16.96 cm), and control (15.67). Based on the LSD test revealed that the treatment C (3:1) was significantly different over all of the treatments and control at $p < 0.05$ while the treatment of B (2: 1), A (1:1) and control was not significantly different of one another at $p < 0.05$ (Table 2).

Table 2. Average primary root length of the corn plant after the intervention of *P. putida* IFO 14796 for 96 h

Treatments	Length of Root (LOR)(cm)
Control	15.67 ^{ns}
A (1 : 1)	17.17 ^{ns}
B (2 : 1)	16.96 ^{ns}
C (3 : 1)	19.53 [*]

*mean difference is significant at $p < 0.05$
ns (non-significantly different at $p < 0.05$)

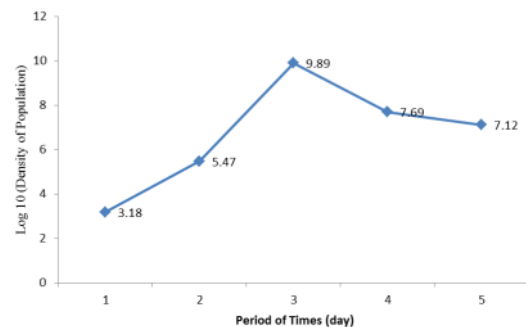


Fig. 1. Population density of *Pseudomonas putida* IFO 14796 in the potato extract-sucrose broth (PESB)

Discussion

Plant growth-promoting rhizobacteria (PGPR) has been able to increase plant growth and crop productivity. One of the important PGPR is *Pseudomonas putida* that has been well known to build the colonization in the root of maize (Neal and Ton, 2013). The present study shows that inoculum of *P. putida* IFO 14796 provides greater benefits to the early corn plant growth especially stimulated the elongation of

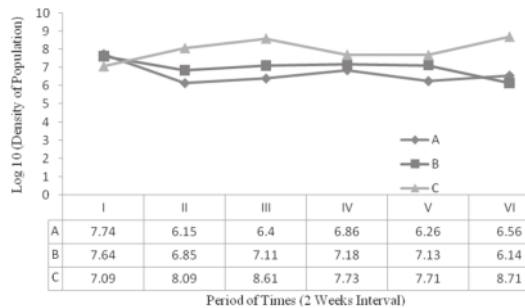


Fig. 2. The survival of *Pseudomonas putida* IFO 14796 for 3 months in the carrier

the primary root without chemical fertilizer application. Likewise, the previously studied show *Pseudomonas* sp. increased the primary, lateral, and semi-l root lengths, and the number of roots, and dry primary roots mass of 5-day-old seedlings (Zerrouk et al., 2016), and total dry root weight (Li et al., 2011). Although they have also shown an increase in shoot and root weight, plant height and plant diameter from bacterial consortium better in mono-inoculum treatments for blue maize plant (Molina-Romero et al., 2017).

In addition, *Pseudomonas polymyxa* CR1 has been produced the phytohormone indole-3-acetic acid (IAA) and utilized the major components of lignocellulose (lignin, cellulose, and hemicellulose) as carbon resources and enhanced the growth of maize (Weselowski et al., 2016) as well as *Pseudomonas aurantiaca* SR1, *Pseudomonas mendocina* Khr2, *Pseudomonas stutzeri* Khr 3 and *Pseudomonas putida* Khr4 (Iqbal Ahmad and Hayat, 2008). Therefore, the ability of *P. putida* IFO 14796 to stimulate the elongation of the primary root corn plant was thought due to the production of siderophores and indol-3-acetic acid (IAA) and several other compounds. Besides that, incubating *P. putida* 14796 in the carrier made from green compost and rice bran that is expected to be a source of carbon to maintain its survival in the rhizosphere for a longer period of time.

Despite *P. putida* can still trigger seedlings under stressful environment such as drought (Liddycoat, et al., 2009), but watering has been carried out every morning and evening to prevent the stress of the bacteria and plant seeds. The weakness of the present study was not monitoring the potency of limiting factors in the rhizosphere, such as salinity, acidity, temperature, and other colonization bacte-

ria. Thus, *P. putida* IFO 14796 is also expected to play an important role in improving the quality of maize grain such as increasing mineral content, vitamins and proteins as well as *Pseudomonas fluorescens* Pf4 which increased grain starch content, especially the digestible components (Berta *et al.*, 2014). In addition, *P. putida* can also reduce Fe³⁺ to Fe²⁺ that is absorbable for plant (Sah Singh and Singh, 2017; Sharma and Johri, 2003; Naz and Bano, 2010). Therefore, it is a potential to apply as PGP for improving the mineral contents of Fe, Ca and Zn in maize grain through biofortification strategies. This is an important role because the mineral ingredient of maize grain was relatively low with the content of Fe(6.2), Ca (19.1), and Zn (9.7 mg/kg) (Galan *et al.*, 2013).

Conclusion

Inoculating of *Pseudomonas putida* IFO 14796 with the density 5.1×10^7 CFU g⁻¹ in sterilized soil can stimulate the elongation of the primary root of the corn plant. This study then proposes to use the *P. putida* IFO 14796 inoculum in biofortification strategies for improving the mineral contents of maize grain.

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Supplementary Data

Analysis of Variance (ANOVA) of the plant biomass, number of roots, and primary root length of corn seeds

	Sum of Squares	Df	ANOVA		
			Mean Square	F	Sig.
W	Between Groups	.000	3	.000	.825
	Within Groups	.001	8	.000	
	Total	.001	11		
RC	Between Groups	.917	3	.306	.627
	Within Groups	4.000	8	.500	
	Total	4.917	11		
RL	Between Groups	43.297	3	14.432	.047*
	Within Groups	27.553	8	3.444	
	Total	70.850	11		

*Mean different is significant at $p < 0.05$

Note: W: (Biomass of the plant); RC (Number of Roots); RL (Length of Roots)

ANOVA and LSD of Length of Roots of Corn Plant

ANOVA					
Roots					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	54.087	3	18.029	5.758	.004
Within Groups	75.143	24	3.131		
Total	129.230	27			

Multiple Comparisons

Dependent Variable: Roots

LSD

(I)	(J)	Mean Difference (I-J)		Std. Error	Sig.	95% Confidence Interval	
		Lower Bound	Upper Bound			Lower Bound	Upper Bound
1	2	-1.586	.946	.107	-3.54	.37	
	3	-1.286	.946	.187	-3.24	.67	
	4	-3.857(*)	.946	.000	-5.81	-1.91	
2	1	1.586	.946	.107	-.37	3.54	
	3	.300	.946	.754	-1.65	2.25	
	4	-2.271(*)	.946	.024	-4.22	-.32	
3	1	1.286	.946	.187	-.67	3.24	
	2	-.300	.946	.754	-2.25	1.65	
	4	-2.571(*)	.946	.012	-4.52	-.62	
4	1	3.857(*)	.946	.000	1.91	5.81	
	2	2.271(*)	.946	.024	.32	4.22	
	3	2.571(*)	.946	.012	.62	4.52	

* The mean difference is significant at the .05 level.

Proliferating of *Pseudomonas putida* IFO 14796 in Potato Extract-Sucrose Broth (PESB)

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