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GROWTH IMPROVEMENT OF TOMATO WITH THE APPLICATION OF BACTERIAL ISOLATES PRODUCING INDOLE ACETIC ACID (IAA) AND PHOSPHATE SOLUBILIZER

Peningkatan Pertumbuhan Tomat dengan Aplikasi Isolat Bakteri Penghasil Asam Indol Asetat dan Pelarut Fosfat

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ABSTRACT

Soil bacteria have important roles in biogeochemical cycle for soil fertility and have been manipulated for ecologically-friendly crop production. The search for beneficial association between microbes and plants for promoting growth and health should be studied for tomato growth improvement. The study aimed to evaluate 19 microbial isolates which produced indole acetic acid (IAA) affecting growth and development of tomato (Palupi variety), and molecularly identify the most effective isolates in improving tomato growth based on 16s rDNA sequences. The experiment was conducted in pots using a complete randomized design with three replications. The parameters observed included plant height, plant dry weight, root length, root dry weight, and fruit fresh weight. The isolates that significantly improved tomato growth were molecularly identified using 16s rRNA sequence. The phenotypic properties such as IAA content and phosphate solubilizing index (PI) of the superior isolates were determined. Results showed that the application of bacterial isolates on tomato significantly increased plant dry weight and fruit yield. From 19 isolates tested, Aj 3.7.1.14 significantly increased plant dry weight, root length, and fruit yield. This isolate produced IAA of about 14.77 ppm and PI of 1.86. Molecular analysis on Aj 3.7.1.14 demonstrated that the isolate had 89% similarity to *Pseudomonas fragi*. The identified *P. fragi* was found to be the most effective isolate for improving tomato growth and fruit yield. Another isolate, *Bacillus amyloliquefaciens* was found to promote root length, root dry weight, and fruit yield. These isolates are potential to be further investigated for field trials

[**Keywords**: bacterial isolate, indole acetic acids, phosphate solubilizer, tomato plant, 16s rDNA]

ABSTRAK

Bakteri tanah memiliki peran penting dalam siklus biogeokimia untuk kesuburan tanah dan telah dimanipulasi untuk produksi tanaman yang ramah lingkungan. Pencarian mikroba yang berasosiasi dan meningkatkan pertumbuhan dan kesehatan tanaman, termasuk tanaman tomat, penting dilakukan untuk menemukan pupuk hayati sebagai substitusi pupuk kimia. Penelitian bertujuan untuk mengevaluasi 19 isolat mikroba penghasil asam indol asetat (IAA) yang meningkatkan pertumbuhan dan hasil tanaman tomat, serta mengidentifikasi secara molekuler isolat yang paling efektif meningkatkan pertumbuhan tomat berdasarkan sekuen gen 16s rDNA. Penelitian dirancang menggunakan rancangan acak lengkap dengan tiga ulangan yang dilakukan menggunakan pot. Parameter yang diamati meliputi tinggi tanaman, berat kering tanaman, panjang akar, berat kering akar, dan berat buah segar. Isolat yang paling efektif meningkatkan pertumbuhan dan hasil tomat diidentifikasi secara molekuler menggunakan sekuen 16 rRNA. Hasil penelitian menunjukkan bahwa aplikasi isolat bakteri secara signifikan meningkatkan berat kering tanaman dan hasil buah tomat. Dari 19 isolat yang digunakan, Aj 3.7.1.14 secara signifikan meningkatkan berat kering tanaman, panjang akar, dan hasil buah. Isolat ini menghasilkan IAA sekitar 14,77 ppm dan indeks pelarutan fosfat 1,86. Berdasarkan analisis sekuen 16 rRNA, isolat Aj 3.7.1.14 mempunyai 89% kesamaan dengan Pseudomonas fragi. *Isolat Aj 3.7.1.14 (*P. fragi*) merupakan isolat yang paling efektif meningkatan berat kering tanaman, panjang akar, berat kering akar, dan hasil buah. Isolat lainnya,* Bacillus amyloliquefaciens *mampu meningkatkan panjang akar, berat kering akar, dan hasil buah. Isolat-isolat tersebut berpotensi untuk diuji lebih lanjut pada kondisi lapangan.*

[**Kata kunci**: isolat bakteri, asam indol asetat, pelarut fosfat, tanaman tomat, 16s rDNA]

INTRODUCTION

Tomato is one of important vegetable crops cultivated all over the world. Tomato fruit has important roles as sources of industrial raw material and nutrition, such as vitamins (A, C, and K), especially lycopene, and carotene Biotin (USDA 2010). Tomato plant has also been used as a model plant for fruit plant research (Kojima et al., 1994; Nitsch et al. 2009). This species was not sufficiently studied for natural sources of biocontrol and/or biofertilizing agents (Romero et al. 2014; Botta et al. 2013). Searching beneficial microbials naturally associated with tomato plant may contribute to the identification of potential candidates of plant growth-promoting characters.

Plants form mutually beneficial associations with microbes. These associations play essential roles in agricultural production system and food safety, and contribute to the environmental equilibrium, stimulate plant growth development, provide resistance to various abiotic and biotic stress factors, improve nutrient acquisition, and protect plants from various soil-borne pathogens (Mendes et al. 2013; Grover et al. 2013; Cho et al. 2015). Microbial phytohormons have been known as plant growth regulator for enhanching metabolism and additionally play a role for defence mechanism against stresses (Egamberdieva et al. 2017). One of important microbial phytohormones, indole acetic acid (IAA), was proven to promote plant growth and development, such as cell division, elongation, and differentiation (Asgher et al. 2015). It was also reported that phytohormones in the group of IAA increase the rate of xylem and root formation; control processes of vegetative growth, tropism, florescence, and fructification of plants; and affect photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to biotic stresses (Bashan et al. 2006; Bashan and de-Bashan 2010).

Combination of IAA and cytokinin levels increases in the seed during its development, concomitant with fruit growth stages where cell division is followed by a cell-expansion phase (Devoghalaere et al. 2012). The role of IAA for resistance to abiotic stresses (e.g. salinity) on tomato has been reported (de la Torre-González et al. 2017). Numerous soil bacteria and fungi also produce phytohormones. It is reported that members of the genera *Azospirillum, Rhizobium, Bradyrhizobium, Enterobacter*, Erwinia and other *Pseudomonas spp*. produced phytohormones which may be potential to alter the physiological aspects of plant, leading to diverse outcomes from pathogenesis to promote plant growth (Goswami et al 2016; Spaepen 2015).

New incentive for plant production is necessary to meet the food quality and quantity for supporting world's population and public health trends, such as the application of plant growth promoting bacteria (Tilman et al. 2002). The support of microbes diversity for plant growth improvement and food production will also provide benefits for environmental sustainability and reduce dependency on chemichal fertilizer, pesticide and fungicide. Researches with growth regulators and hormones associated with nutrient aim to accelerate the development of plants. The application of such elements in the early stages of plant development can stimulate root growth which would provide faster recovery after a period of water stress; greater resistance to pests, diseases and nematodes; and more rapid and uniform establishment of plants (Nassal et al. 2018; du Jardin 2015; Zhao 2012; Bashan and de-Bashan 2010; Spaepen and Vanderleyden 2011).

For increasing tomato yield, most farmers in Indonesia still rely on chemical fertilizers. However, it is reported that excessive use of inorganic fertilizers in the long run can decrease the level of productivity and soil fertility. In addition to negatively impacting soil fertility, the problems facing farmers today are the increasing scarcity and price of inorganic/chemical fertilizers (Darwis and Supriyadi 2013; Suryana et al. 2016). It is reported that innoculation of *Pseudomonas sp*. RU49 in soil associated with the increased posphatase activity in the soil and improved tomato plant growth (Nassal et al. 2018). The objectives of this study were to (1) evaluate the effectiveness of 19 microbial isolates which have P solubilizer and plant growth regulator characters in enhancing plant growth and yield of tomato (Palupi variety) grown in pots, and (2) molecularly identify the most effective isolates in improving tomato growth based on 16s rDNA sequences.

MATERIALS AND METHODS

Bacterial Isolates and Maintenance

The experiment was conducted in the laboratory and greenhouse in Microbiology Conservation of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor, from April to September 2016. The favorite tomato variety (Palupi) was used in this experiment.

Nineteen bacterial isolates were used in this study (Table 1). The isolates are belonged to and have been deposited in the BB Biogen Culture Collection of ICABIOGRAD.

The isolates were stored in freeze dryed form for a long term storage at the BB Biogen Culture Collection, and were recultured using Nutrient Agar (NA) or Nutrient Broth (NB) media and incubated at 30 ^{o}C for the purposes of providing fresh bacterial cultures prior use of the experiments.

In vitro Indole Acetic Acid (IAA) Assay

IAA was assayed by following the method of Glickmann and Dessaux (1995). About 1 ml of culture (in NB medium at 30 ^{o}C for 24 hours in the shaker incubator at 200 rpm) and 1 ml L-tryptophan were added into 10 ml of minimal medium containing 1.36 g KH_2PO_4, 2.13 g Na_2HPO_4, 0.5 g $MgSO_4.7H_2O$, and 100 ml trace elemen containing 700 mg $CaCl_2$, 300 mg $FeSO_4$, 20 mg $MnSO_4$, 40 mg $CuSO_4$, 20 mg $ZnSO_4$,

Tabel 1. Bacterial isolates used in this study and their origins.

	Isolate code	Source of bacterial isolates
1	FB Endo 26	Endophytic bacteria from rice plant
2	FB Endo 68	Endophytic bacteria from rice plant
3	FB Endo 79	Endophytic bacteria from rice plant
4	FB Endo 80	Endophytic bacteria from rice plant
5	FB Endo 95	Endophytic bacteria from rice plant
6	Endo 5 Bandung	Endophytic bacteria
7	Endo 113	Endophytic bacteria from rice plant
8	FB Endo 135	Endophytic bacteria from rice plant
9	FB Endo 137	Endophytic bacteria from rice plant
10	FB Endo 140	Endophytic bacteria from rice plant
11	Azm 1.7.2.12	Rhizosphere bacteria from rice plant (mutated)
12	Aj 1.9.3.2	Rhizosphere bacteria from rice plant
13	Aj 3.7.1.14	Rhizosphere bacteria from rice plant
14	Aj 5.2.5.1	Rhizosphere bacteria from rice plant
15	Azm 5.7.2.1	Rhizosphere bacteria from rice plant (mutated)
16	Aj Bandung 6.4.12	Rhizosphere bacteria from rice plant
17	Aj 18.3.1	Rhizosphere bacteria from rice plant
18	Aj 20.1.4	Rhizosphere bacteria from rice plant
19	Azoto 2-1	Rhizosphere bacteria

3 mg H_3BO_3, 7 mg $COCl_2.6H_2O$, 4 mg $Na_2MoO_4.H_2O$, and 1 ml H_2SO_4 in 1 liter medium. The culture was then incubated at 30 ^oC, 200 rpm, for 2 x 24 hours and centrifuged at 10,000 rpm at 4 ^oC for 10 minutes. A total of 2 ml supernatant was added and homogenized with 4 ml Salkwoski solution using vortex, and then incubated at room temperature for 1 hour. Salkowski's reagent is a 35% $HClO_4$ solution containing 10 mM $FeCl_3$, and when mixed with IAA, tris-(indole-3-acetate) iron (III) complex is formed to display pink coloration (Kamnev et al. 2001). The IAA concentration of the mixture was then measured in triplicate using Hitachi U2.800 UV-vis at 530 nm compared to IAA standard from Sigma Aldrich.

P Solubilization Assay

The P solubilization characteristic was assayed in Pikovskaya's plate containing 10 g glucose, 5 g calcium phosphate, 0.2 g sodium chloride, 0.2 g potassium chloride, 0.1 g magnesium sulphate, 2.5 mg manganese sulphate, 2.5 mg Ferro (II) sulphate, 0.5 g ammonium sulphate, and 0.5 g yeast extract per liter medium (pH 8.5) (Chen et al. 2006). The culture was then incubated at 30 °C for 7 days and colony with a clear halo was marked positive for phosphate solubilization activities. P solubilizing index was measured as follow:

$$\text{P solubilizing index} = \frac{\text{Diameter of clear zone}}{\text{Diameter of Colony}}$$

All of the measurements were done in three replications.

Bacterial Isolate Identification Activity

Gram Reaction Determination of Bacterial Isolates.

KOH (3 %) was used to determine the Gram reaction of the isolates. About 300 µl of KOH (3 %) was mixed with a loopful of bacterial isolates above a glass slide. Mixtures producing a sticky slime type in less than 60 seconds was determined as gram negative bacteria (Moaledj 1996).

Bacterial Species Identification Using 16S Rdna Sequences.

Three of the selected bacterial isolates which improve tomato growth and fruit yield were choosen for species identification. Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega, USA) and visualized in Geldoc UV transilluminator after electrophoresis using 1 % agarose electrophoresis. DNA concentrations were measured using NanoDrop 2000 (Thermo Scientific, USA). The 16s rDNA gene fragment was amplified using primers (63F and 1387R) at Polymerase Chain Reaction (PCR) machine, using the following PCR program: pre-denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 45 seconds, annealing at 53 °C for 45seconds, and elongation at 72 °C for 3 minutes. Cycles was repeated for 20 times, followed by elongation at 72 °C for 7 minutes and final incubation at 4 °C. Sequencing of the 16s rDNA gene fragment was done commercially (Firstbase, Singapore) through PT Genetika Science Indonesia. The sequence data were then analyzed using the Basic Local Alignment Search Tool Nucleotide (BLASTN) program from National Center for Biotechnology Information (NCBI) site.

Microbial Application, Planting, Plant Growth Evaluation

Microbial aplication was done twice, as seed treatment and foliar spray. For seed treatment, tomato seed was sterilized with 2 % NaOCl for 5 minutes, followed by soaking in ethanol 70 % for 5 minutes, and rinsed with sterile water 5 times. The first application, the sterile seed was then inoculated by immersing with bacterial isolate with cell density of 10^7 cells ml^{-1} in 30 ^oC shaker incubator for 1 hour. The seeds were then dried and transferred into sterile filter papers and incubated at 30 ^oC for 24 hours. The tomato seedling was prepared by transferred the inoculated seeds into plastic tray containing soil until seedling about 2 weeks old.

Second bacterial inoculation was applied by foliar spraying at 30 days after planting (DAP). Each bacterial isolate was recultured in NA medium at 30 °C for 24 hours. For the inoculation application, each isolate was cultured in the petridish, then the cells were collected and resuspended into 100 ml solution containing 90 ml 0.1 M $MgSO_4$ and 10 ml of 1 % CMC with cell population of about 107 cells ml^{-1}. This solution was then used as bacterial carrier for better survival during application (unpublished). Control treatment was made by spraying the plant with 100 ml medium containing a mixture of 90 ml of 0.1M $MgSO_4$ and 10 ml of 1 % CMC.

Two tomato seedling per pot were grown in pot containing 6 kg soil medium mixture with dung manure. The experiment was carried out using a complete randomized design (CRD) with three replications conducted at ICABIOGRAD greenhouse. Fertilizers were applied before planting as for tomato planting recommendation (125 kg ha^{-1} urea, 300 kg ha^{-1} ZA, 250 kg ha^{-1} TSP and 200 kg ha KCl^{-1}). Other plant maintenances such as irrigation, fungicide and pesticide applications were done as needed.

Parameters observed included plant height, per plant whole plant dry weight, root length, root dry weight, and fruit fresh weight. Plant height was measured and harvested at 90 days after planting (dap). The roots were separated from the plant and washed with tap water and air dried before dried in the incubator. The fruits were harvested after red color was observed.

Data Analysis

The data were subjected to analysis of variance (ANOVA) and the treatment mean values were compared by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ for significance level using Statistical Package Version 17.0 (SPSS 17.0) program (IBM Corporation 2009).

Results and Discussion

IAA Production and Phosphate Solubilazion Properties

Our finding showed that all of 19 isolates produced IAA with different levels of concentrations ranging from 7.63 ppm to 40.8 ppm. The highest value of IAA concentration (40.8 ppm) was shown by isolate FB Endo 135, while the lowest (7.63 ppm) was shown by isolate Azm 1.7.2.12 (Table 2).

All 19 isolates were capable of dissolving $Ca_3(PO_4)_2$ in the Pikovskaya medium as the isolates form a clear zone around the colony. The P index resulted by the 19 isolates ranged from 1.3 to 3.0 (Table 2). The highest IP index was 3.0 demonstrated by isolate Azm 1.7.2.12, indicating its highest capability for dissolving unsoluble P. The lowest P index was 1.32 demonstrated by isolate Aj 18.3.1 (Table 2).

Previous reports showed that over 80 % of the bacteria isolated from the rhizosphere are capable of synthesizing IAA. Higher concentration of IAA of 106 ppm was produced by *Pseudomonas sp*. as reported by Ali et al. (2009). The *Pseudomonas sp*. was isolated from rhizosphere. In the optimum concentration, IAA was reported responsible for division, extension, and differentiation of plant cells and tissues (Bashan et al. 2006; Bashan and de-Bashan 2010).

Another report on rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia, showed that 46.52 % isolates are capable of solubilizing inorganic phosphate with solubilization index ranged from 1.1 to 3.05. However, there was no report on their effect on plant growth (Elias et al 2016).

Tabel 2. Mean values of P index, IAA production and gram classification of the bacterial isolates used in this study.

	Isolates	P index	IAA production (ppm)	Gram reaction classification[1)]
1	FB Endo 26	2.17	23.91	+
2	FB Endo 68	1.30	25.83	+
3	FB Endo 79	1.67	19.32	+
4	FB Endo 80	1.33	15.37	+
5	FB Endo 95	1.60	15.78	-
6	Endo 5 Bandung	2.83	21.25	+
7	Endo 113	1.58	20.19	+
8	FB Endo 135	1.46	43.8	+
9	FB Endo 137	1.71	13.68	-
10	FB Endo 140	1.70	10.95	+
11	Azm 1.7.2.12	3.00	7.63	+
12	Aj 1.9.3.2	1.70	27.82	+
13	Aj 3.7.1.14	1.86	14.77	-
14	Aj 5.2.5.1	1.52	28.56	+
15	Azm 5.7.2.1	2.60	12.18	+
16	Aj Bandung 6.4.12	1.67	13.29	+
17	Aj 18.3.1	1.32	27.82	+
18	Aj 20.1.4	2.21	23.29	-
19	Azoto 2-1	2.28	14.3	+

*+ = gram positive bacteria; - = gram negative bacteria

Effect of Bacterial Isolate Application on Growth and Yield of Tomato Plant

Results showed that plant dry weight, root length, root dry weight, and fruit yield were significantly affected by bacterial application (Table 3). The highest tomato fruit yield (735.12 g per pot) was achieved by isolate Aj 3.7.1.14. The isolate also improved plant dry weight and root length (Table 3). Isolate Aj 3.7.1.14 produced moderate IAA and moderate P index compared to other isolates tested in the study. However, this isolate demonstrated the highest effect on tomato growth and yield. This indicates that the combination of moderate IAA production and moderate P index shown by isolate Aj 3.7.1.14 gave the best tomato growth and yield. The highest IAA concentration was resulted by isolate Endo 135 and the highest P index was demonstrated by isolate Azm1.7.2.12, but these isolates did not significantly affect tomato growth and yield.

From Tables 3 and 4, Aj 3.7.1.14 was the most promising isolate for tomato plant growth and yield as the isolate showed significant effects on the three observed parameters (i.e. plant dry weight, root length, and fruit yield). This isolate was obtained from rice plant's rhizospehre (Table 1) and applied as seed treatment and foliage treatment. A similar study on *Triticum aestivum* using *Pseudomonas sp*. isolated from rhizosphere produced high concentration of IAA (106 ppm), but this isolate did not give the best effect on *T. aestivum* growth compared to other isolates tested in this study (Ali et al. 2009).

Isolate Aj 3.7.1.14 was identified as *Pseudomonas fragi* with the maximum identity of 98% based on the 16s rDNA sequence similarity, while isolate FB Endo 80 identified as *Bacillus amyloliquefaciens* (Table 4). The later isolate showed significant effect on root dry weight As previously described Aj 3.7.1.14 was isolated from rice rhizosphere and significantly improved tomato growth and fruit yield.

Based on P index and IAA concentration characterization results, the most effective isolate (Aj 3.7.1.14) did not produce the highest value of P index as well as IAA concentration. The highest IAA production was demonstrated by isolate Endo 135 and the highest P index was shown by isolate Azm 1.7.2.12. This result suggested that isolate Aj 3.7.1.14 should have other important characteristics which effectively enhance tomato growth and fruit yield. Similar results were demonstrated by Ali et al. (2009) who reported that *Pseudomonas sp*. As-17 isolated from rhizosphere produced high IAA concentration of about 106 ppm, but its application on *T. aestivum* under *in vitro* conditions using sterilized sand medium did not show highest root length, shoot length, and 100 seeds weight (Ali et al. 2009). This study did not report

Tabel 3. Effect of bacterial isolates on tomato growth and yield.

Code	Bacterial isolates	Plant height (cm)	Plant dry weight (g pot^{-1})	Root length (cm)	Root dry weight (g pot^{-1})	Fruit fresh weight (g pot^{-1})
A1	FB Endo 26	126.33	43.59bcde	17.66defg	10.07ab	96.84b
A2	FB Endo 68	117.66	47.95bcd	18.33cdef	6.99ab	174.74b
A3	FB Endo 79	128.33	37.80bcde	16.66defg	6.64ab	289.70b
A4	FB Endo 80	139	43.96bc	18.50abc	15.30a	210.75b
A5	FB Endo 95	128	48.74abc	24bcd	11.72ab	234.04b
A6	Endo 5 Bandung	126	48.10bcd	24.66abc	6.27ab	183.32b
A7	Endo 113	128.66	41.97cd	22.66bcde	12.80ab	218.46b
A8	FB Endo 135	133.66	44.92cd	23.66bcde	9.23ab	234.55b
A9	FB Endo 137	120.66	32.40de	21bcde	10ab	280.83b
A10	FB Endo 140	122.33	34.70cde	27.83abc	9.07ab	256.18b
A11	Azm 1.7.2.12	121.33	44.51bcd	12.33fg	11.52ab	218.52b
A12	Aj 1.9.3.2	141.33	38.72bcd	30.66ab	2.43b	371.39ab
A13	Aj 3.7.1.14	132.33	53.30a	31.66a	11.85ab	735.12a
A14	Aj 5.2.5.1	131	28.83e	26.00bcde	9.32ab	151.40b
A15	Azm 5.7.2.1	132.33	54.29a	21.83bcde	8.19ab	212.90b
A16	Aj Bandung 6.4.12	139,66	45.22bcd	13.66efg	6.14ab	234.84b
A17	Aj 18.3.1	119	39.29bcd	30ab	5.98ab	250.24b
A18	Aj 20.1.4	130	53.78a	16defg	5.64b	177.25b
A19	Azoto 2-1	138.33	42.34bcd	10.66g	7.14b	143.31b
A21	Neg. control	123.66	41.85bcde	29.66ab	7.04b	156.06b
	F. value	1 ^{ns}	2.115^{s}	4.702^{s}	0.843^{s}	0.933 ^{s}

Note:
Values in a row followed by the same letters are not significantly different (P =0.05) according to Duncan's Multiple Range Test (DMRT).
s = Significant; ns = not significant.

Tabel 4. Bacterial isolates significantly improved tomato growth and yield in which their species identification was determined based on 16s rDNA sequences.

Growth and yield parameters improved	Isolate codes	Species identification based on 16s rDNA sequences	Accession number	Maximum identity of the isolates to the NCBI database*
Plant dry weight	Aj 3.7.1.14	*Pseudomonas fragi*	NR_024946.1	98%
	Aj 20.1.4	*Pseudomonas fragi*	NR_074540.1	95%
Root length	Aj. 3.7.1.14	*Pseudomonas fragi*	NR_024946.1	98%
	Aj 1.9.3.2	*Bacillus amyloliquenfaciens*	NR_117946.1	99%
Root dry weight	FB Endo 80	*Bacillus amyloliquenfaciens*	NR_075005.1	99%
	Endo 113	*Bacillus cereus*	NR_074540.1	98%
Fruit yield	Aj 3.7.1.14	*Pseudomonas fragi*	NR_024946.1	98%
	Aj 1.9.3.2	*Bacillus amyloliquenfaciens*	NR_117946.1	99%

*The maximum identity was determined based on the percentage of 16s rDNA sequence similarity resulted from the alignment of 16s rDNA sequences of the tested isolates compared to those existed in the NCBI data base.

the effect of isolate application on fruit yield that was done in our study. Another report on the application of microbes (*Penicilium brevicompactum, Trichoderma atroviride, Psedomonas marginalis,* and *P. putida*) on tomato plant resulted plant growth stimulation, most likely, due to the synergic result of numerous modes of action exhibited by each microorganism tested. This included a regulation in the concentration of IAA in the rhizosphere and a regulation of the concentration of ethylene within the roots (Gravel et al. 2007). Gravel et al. (2007) showed that microbial application did not significantly increase tomato fruit yield. Elyazied and Abou-aly (2011) reported that phosphate solubilizing microbial application in combination with rock phosphate positively affected tomato fruit yield as a result of increasing dehydrogenase and phosphatase activity.

Plant-*Pseudomonas* interaction was recognized both as saprophyte and parasite on plant surface and inside plat tissues. Some *Pseudomonas* increase the incidence of damage to host tissues through ice nucleation (Lindow and Brandl 2003). Many plant-associated *Pseudomonas* promote plant growth by suppressing the growth of pathogenic micro-organisms. Other plant-associated *Pseudomonas* inhibit plant growth and cause disease symptom development (Preston 2004). From this result, isolate *P. fragi* (Aj. 3.7.1.14) was proven to be beneficial *Pseudomonas* for tomato growth and yield (i.e. plant dry weight, root length, and fruit yield). Please note that our experiment did not use sterile soil medium, and, therefore, the effect of microbial population in the soil may also have influence on the study results. However, both of the microbial populations are predicted to have positive association for enhancing tomato growth and yield.

In this experiment, Aj 3.7.1.14 (*P. fragi*) isolated from rhizosphere of rice plant demonstrated better effect on tomato growth and yield compared to that of endophytic isolate Endo 80 (*B. amyloliquefaciens*) with two methods of applications (seed and foliar treatments). Moreover, we found that the isolate showing the highest IAA production and highest phosphate solubilization capacity did not show the best effect on tomato growth and yield. The best tomato growth and yield were demonstrated by isolate showing combination of moderate IAA production and medium phosphate solubilization capacity. The results of this study suggested that the compatible plant-bacteria association in improving plant growth and yield might be unique to specific plant species or variety.

CONCLUSION

Plant growth and fruit yield of tomato (Palupi variety) were improved by bacterial isolate application Isolate Aj 3.7.1.14, identified as *P. fragi* producing moderate IAA and moderate phosphate index, showed the highest effect on plant growth (plant dry weight and root length) and fruit yield of tomato, Palupi variety compared to other bacterial isolates tested. Another isolate (Endo 80), identified as *B. amyloliquefaciens* showed significant effect on root length, root dry weight, and fruit yield. The latter isolate might have other important characteristics that can be the subject for further investigation.

Further field trial and application method, e.g. isolate, formulation should also be investigated to obtain more comprehensive information on the superior bacterial isolates capable of improving tomato growth and fruit yield.

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