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Plant growth-promoting properties of free-living diazotrophic rhizobacteria from Tangerine (*Citrus reticulate L.*) var Batu 55

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ABSTRACT

Aims: Microbial cultures with multi-biological activities in increasing plant growth were developed to be an alternative way to reduce dependency on chemical fertilizer and to support plants productivity. The aims of this study were to characterize the potency of Tangerine var. Batu 55 diazotroph rhizobacteria as Plant Growth Promoting Rhizobacteria (PGPR) agents and to identify diazotroph rhizobacteria with multi-biological activities especially the activity of nitrogen fixation, ammonia (NH_3) production, phosphate solubilizing, and Indole Acetic Acid (IAA) production.
Methodology and results: A total of 21 nitrogen-fixing bacteria (diazotroph) were isolated from Tangerine rhizosphere soil. Screening of PGPR isolates candidates were performed by *in vitro* assays consist of phytohormone *Indole Acetic Acid* (IAA) production, ammonia production, and phosphate-solubilizing assay. Candidates of PGPR isolates were identified based on 16S rDNA sequences. The result revealed that three isolates (Dbs 1, Dbs 2, and Dbm 3) had multi-biological activities. Isolates of Dbs 1, Dbs 2, and Dbm 3 capable producing ammonia up to 10 µg/mL; 9.1 µg/mL; and 3.8 µg/mL, activity of IAA production were 30.08 µg/mL; 24.68 µg/mL; and 190.77 µg/mL, activity of phosphate solubilizing were 11.3 µg/mL; 8.6 µg/mL; and 2.2 µg/mL, respectively. Based on 16S rDNA, Dbs 1, Dbs 2, and Dbm 3 were identified as *Acinetobacter schindleri*, *Pseudomonas syncyanea*, and *P. moraviensis*, respectively. Based on our knowledge, this is the first report *P. syncyanea* was exhibited plant growth-promoting properties.
Conclusion, significance and impact of study: Candidates of PGPR isolates could be alternative PGPR agents, but still need to evaluate the effect of three PGPR isolates application on citrus plant growth.

Keywords: 16S rDNA sequences, diazotroph bacteria, plant growth promoting bacteria, tangerine Batu 55 rhizosphere

INTRODUCTION

Tangerine (*Citrus reticulata* L.) var. Batu 55 is one of the popular local citrus varieties in East Java, Indonesia which has good quality in both appearance and taste. Tangerine Batu 55 have high production potency up to 100 kg/tree and the production can reach tens of years. Strategies of plants cultivation techniques and proper maintenance are necessary to maintain citrus productivity (Sugiyatno, 2014; Setiono, 2015; Sugiyatno, 2015). Biofertilizers utilization can be an effective way to reduce the dependence on chemical fertilizers in the cultivation of citrus plants (Hanapi, *et al*., 2013. Hoe *et al*., 2015).

Nitrogen is an essential macronutrient for plant growth. Biological nitrogen fixation is the process that converts the atmospheric nitrogen into ammonia (NH_3) by diazotroph bacteria which is a microorganism that has the ability to fix atmospheric nitrogen then convert it into a usable form for plants. Several studies found that many strains of free-living or non-symbiotic diazotroph bacteria also have potency as Plant Growth Promoting Rhizobacteria (PGPR). PGPR are functional microorganisms with promoting plant growth activity through various mechanisms. Diazotroph bacteria also able to promote plant growth through one or more mechanisms of biological activity, for example, phosphate solubilization, phytohormone production (eg, auxin, ethylene, and cytokines), siderophores production, induce mechanisms of systemic resistance (ISR) to host plants, and potential as a biocontrol against pathogens. In recent years, many studies have been reported genus of bacteria with plant growth promoting mechanisms including *Acinetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhizobium*, *Serratia* (Thakuria *et al*., 2004; Gupta *et al*., 2005; Kumar *et al*., 2008; Ahemad and Khan, 2011). A recent report showed that nitrogen-fixing bacteria such as *Bacillus* sp., *Klebsiella* sp.

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and *Microbacterium* sp. which were isolated from various rice cultivars in Korea had the ability to produce IAA, siderophore, and pathogen inhibition (Ji *et al*., 2014). Another report showed that nitrogen-fixing bacteria *Pseudomonas* sp. RFNB3 had multi-biological activities including mineral solubilizing activity, IAA hormone, salicylic acid, ACC deaminase, and siderophore production which resulted in red pepper growth enhancement under greenhouse experiments (Islam *et al*., 2013).

Application of PGPR with multi-biological activities is more efficient for biofertilizer formulation. Initial screening is an essential step in order to select an efficient PGPR agents, so in this research, we will focus on screening and characters studies of PGPR candidates which has been obtained (Sevilla and Kennedy, 2000; Prashant *et al*., 2009; Gupta *et al*., 2012). The aims of this research were (1) to study the potency of diazotrophs bacteria from Batu 55 rhizosphere for their multi-biological activities (2) to characterize and identify based on 16S rDNA sequences of diazotrophs bacteria with multi-biological activities.

MATERIALS AND METHODS

Sample collection and isolation of diazotroph bacteria

The rhizospheric soil was collected from Organic Tangerine field in Kungkuk Village, Batu City,East Java, Indonesia (S 07.83274°, E 112.52509°). Diazotroph bacteria were isolated by modified method of Baldani *et al*. (2014). Rhizospheric sample (25 g) was dissolved in 225 mL of saline solution (0.85% NaCl) and shaken for 2 h at rotary shaker. Non-symbiotic diazotroph bacteria were isolated using serial dilutions method (10^{-1} – 10^{-6}). Each of aliquot (0.1 mL) was inoculated by stabbing into 10 mL of N-free semi-solid medium (pH 6.5 – 7.2) which consist of (g/L): KH_2PO_4 (0.5); $FeCl_3.6H_2O$ (0.015); $MgSO_4.7H_2O$ (0.2); NaCl (0.1); DL-Malic Acid (5); KOH (4.8); Bromothymol blue 0.1% (v/v); and agar (3) then incubated for 7 days at 28 °C. Positive results were shown by the formation of the pellicles in the subsurface of the medium and change of medium color from green to blue. A loopful of pellicles in the highest dilution tube was transferred into a fresh N-free semi-solid medium supplemented with 0.05 g/L yeast extract for purification using spread plate methods. A single colony from purification plates was streaked onto N-free slant and characterized based on morphology and biochemical assays.

Screening for diazotroph bacteria on ammonia production

Accumulation of ammonia was detected using Ammonia Test Kit by a modified method of Iwata *et al*. (2010) as primary screening. A number of isolates with highest activity at this primary screening will be further investigated for other biological activities. Ammonia accumulation was also quantified using Nessler methods. Five milliliters of each strain (107 cells/mL) was grown in 50 mL N-free broth and incubated at 28 °C for 7 days. The uninoculated medium was used as a control. Two milliliters of bacterial suspension were treated with 0.01 mL of $ZnSO_4$ solution and 2.5 µL of 2N NaOH. The treated suspension was incubated for 30 min until flocs precipitate formed and the suspension became colorless or clear. The suspension was centrifuged at 10000 rpm for 10 min, added with 1 mL of Nessler Reagent to 1 mL supernatant and diluted to 10 mL with distilled water. The suspension was incubated for 30 min until the development of yellow color which is an indicator of ammonia production. The absorbance of samples was measured at 425 nm. The quantity of ammonia was determined based on ammonia standard curve (Ahmad *et al*., 2008; Agbodjato *et al*., 2015).

Bioassay of indole-3-acetic acid (IAA) production

Five milliliters of each strain (10^7 cells/mL) was grown in 50 mL Tryptic Soy Broth (TSB) supplemented with 2% (v/v) L-Tryptophan (1 µg/mL) and incubated at 28 °C. The uninoculated medium was used as a control. Two milliliters of bacterial suspension were harvested every day up to 4 days incubation to quantify of IAA production of each strain. The bacterial suspension was centrifuged at 10000 rpm for 10 min, then 2 mL of Salkowski Reagent was added to 1 mL of supernatants and incubated for 30 min in dark condition until the development of pink color which is an indicator of IAA production. The absorbance of samples was measured at 530 nm. Quantification of IAA was determined based on IAA standard curve (Khalid *et al*., 2004).

Bioassay of phosphate solubilizing bacteria

Phosphate solubilizing activity of bacteria was performed by the modified method of Chauhan *et al*. (2014). Five milliliters of each strain (10^7 cells/mL) was grown in 50 mL Pikovskaya Broth (pH 7.0) supplemented with 0.5% Tricalcium Phosphate (TCP) and incubated at 28 °C. The uninoculated medium was used as a control. Ten milliliters of bacterial suspension were harvested up to 9 days incubation to quantify of phosphate solubilized of each strain and to measure the pH of the medium. Two milliliters of bacterial suspension were centrifuged at 10000 rpm for 10 min, then 10 mL of chloromolibdic reagent and 0.1 mL of chlorostannous acid was added to 1 mL of supernatant. The suspension was diluted to 25 mL using distilled water, homogenized, and incubated for 10 min. The absorbance of samples was measured at 690 nm. Quantification of phosphate solubilized was determined based on phosphate standard curve.

Identification of PGPR based on 16S rDNA

Bacterial chromosomal DNA was extracted using a modified protocol Soil i-Genomic DNA Extraction Mini Kit. The sequence of 16S rDNA was amplified using universal primers 27f (5 '-GAG AGT TTG CTG GCT ATC CAG- 3')

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and 1492r (5 '-CTA CGG CTA TGT CCT TAC GA- 3 '). Polymerase Chain Reaction (PCR) programs for 16S rDNA amplifications consist of: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation (94 °C for 30 sec), annealing (55 °C for 30 sec), elongation (72 °C for 90 sec), and final elongation at 72 °C for 5 min) (Wilson *et al*., 1990; Wang *et al*., 2011; Mihalache *et al*., 2015). Amplicons of 16S rDNA were purified and sequenced in First BASE, Malaysia. The 16S rDNA sequences of isolates were aligned with reference strains using the MEGA V.6 program. Phylogeny tree was constructed and inferred with Neighbor-joining algorithm with Tamura-Nei model using 1000 replicates bootstraps

RESULTS AND DISCUSSION

Morphological and biochemical characters of selected PGPR isolate

Rhizospheric soil was collected from the organic field inorder to get selected isolates which have potency as PGPR agents with various activities for promoting citrus plant growth. From 21 isolates obtained from rhizosphere soil of Tangerine Batu 55, three isolates (Dbs 1, Dbs 2, and Dbm 3) were selected based on their ability to accumulate ammonia from biological nitrogen fixation at primary screening as presented in Table 1. All phenotypic characters of selected isolates as PGPR were presented in Table 2.

Activity of IAA production, P solubilizing, and NH_3 production by selected PGPR isolate

Based on the experiments, each isolate was able to produce ammonia with varies concentration (Figure 1a). Dbs 1 isolate tends to produce ammonia with higher concentrations, whereas the Dbm 3 isolate had the lowest activity during 7 days of incubation. The optimum conditions of Dbs 1 and Dbm 3 isolates to produce ammonia were on day 3 incubation (10 µg/mL and 3.8 µg/mL respectively), whereas Dbs 2 isolate was faster to produce ammonia (9.1 µg/mL) after 24 h of incubation. Compared with other studies, diazotroph isolates in this study have higher ammonia production activity. PGP strain BK21 isolate which is isolated from Haryana soil, India is able to produce ammonia up to 2.561 µg/mL (Kumar *et al*., 2013). *Azotobacter chroococcum* that grown at 30 °C and 35 °C produce ammonia about 4.6 mg/L and 6 mg/L, respectively (Saribay, 2003).

Based on the ability of IAA hormone production revealed that all diazotroph isolates were able to produce IAA with varying concentrations (Figure 1b). Dbm 3 isolate can produce IAA hormone with the highest concentration during 4 days of incubation. The optimum production of IAA hormone by Dbm 3 was reached 190.77 µg/mL on 48 h of incubation, while Dbs 1 and Dbs 2 isolates were 30.08 µg/mL and 24.68 µg/mL respectively on 96 h of incubation. Dbm 3 isolate had a similar capability with IAA-producing bacteria AUX 53 isolate from wheat rhizosphere that showed the optimum production of IAA reaches 210 µg/mL using 1% tryptophan in the bacterial growth medium (Aziz *et al*., 2015).

The difference of IAA concentration is due to differences of bacterial species and the concentration of tryptophan which added to the bacterial growth medium as an IAA precursor. Tryptophan is an important precursor compound to accelerate the synthesis of IAA that naturally obtained from plant roots exudate which is absorbed by bacteria. The addition of 1 mg/L tryptophan in the medium increased IAA production by Klebsiella K8, K17, K23, and K30 to 2.2 times greater than without the addition of tryptophan (Sachdev *et al*., 2009; Raut *et al*., 2017).

Based on the experiment results showed that Dbs 2 isolate was able to dissolve phosphorus (P) faster than Dbs 1 and Dbm 3 isolates, but Dbs 1 isolate had the highest potency than the other isolates (Figure 1c). The optimum condition of Dbs 1 isolate in dissolved P was on the day 5 (11.3 µg/mL) but on the day 7 and 9, the soluble P concentration decreased significantly. The optimum condition of Dbs 2 isolate to dissolve P was on the day 2 (8.6 µg/mL), the concentration remained stable until the day 5 then decreased slightly on the day 9 (6.7 µg/mL). Dbm 3 isolate has the lowest ability to dissolve P which only 0.3 µg/mL on the day 2 and the concentration continues to increase slightly on day 7 to 2.2 µg/mL and decrease at day 9.

Table 1: Ammonia accumulation of each diazotroph bacteria at primary screening.

Isolate	Ammonia production	Isolate	Ammonia production	Isolate	Ammonia production
Dbs 1	+++	Dbs 8	+	Dbm 1	+
Dbs 2	++	Dbs 9	+	Dbm 2	+
Dbs 3	+	Dbs 10	+	Dbm 3	+++
Dbs 4	+	Dbs 11	+	Dbm 4	+
Dbs 5	+	Dbs 12	+	Dbm 5	+
Dbs 6	+	Dbs 15	+	Dbm 6	+
Dbs 7	+	Dbs 16	+	Dbm 10	+

+++ = 0.6 – 1 µg/µL, ++ = 0.1 – 0.5 µg/µL, + = less than 0.1 µg/µL ammonia accumulation in culture.

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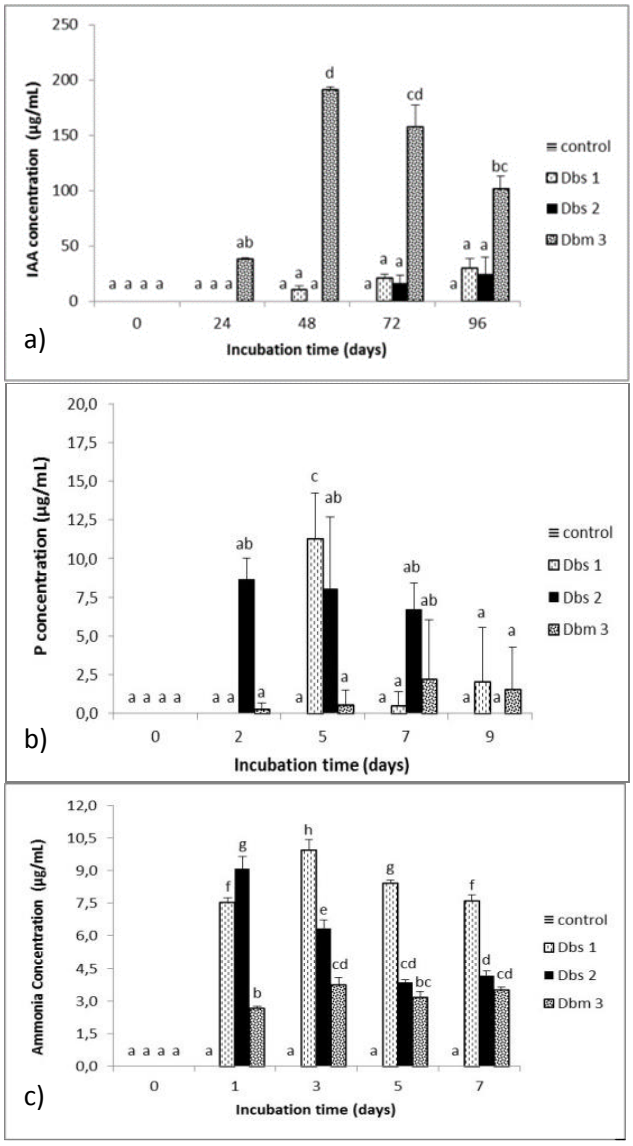

Figure 1: The potency of PGPR to a) produce IAA b) P solubilizing c) and NH_3 production by selected PGPR isolate.
Data were expressed as the mean ± standard deviation of three replicates experiment using Two-way ANOVA test at α = 0.05. Different notations indicate that a significant difference among treatments ($p < 0.05$).

The acidity (pH) of the media was measured before and after the incubation time to determine the effect of P dissolution. Based on Table 3, control treatment did not significantly decrease the pH value ($p > 0.05$), while the growth media of Dbs 1, Dbs 2, and Dbm 3 isolates decrease the pH value significantly ($p < 0.05$) until final pH became slightly acid. The changes of acidity of media in each diazotroph bacteria is due to the low of P dissolution activity. In the previous studies, *Pantoea agglomerans* and *Burkholderia anthina* with P dissolving activity of about 600 µg/mL were able to change the initial pH medium from pH 7 to 3.83 and 3.82, respectively (Walpola and Yoon, 2013).

Potency of selected PGPR isolate as PGPR agents

Characters of PGPR activity from each isolate indicated that the highest ammonia production activity was performed by *Acinetobacter schindleri* isolate Dbs 1 and *Pseudomonas syncyanea* isolates Dbs 2, the highest phosphate solubilizing activity was performed by *A. schindleri* isolate Dbs 1, and the highest IAA production activity was performed by *P. moraviensis* isolate Dbm 3, indicating that each isolate has different main mode of action in increasing plant growth. To optimize the application of PGPR bacteria for enhancing plant growth it is necessary to ascertain the potency of each isolate in consortium culture and association assay to determine the effect of antagonistic bacterial growth due to the presence of other microbes (Cattelan *et al*., 1999; Wong *et al*., 2014).

Phylogenetic tree of selected PGPR isolate based on 16S rDNA sequences

Phylogeny tree of diazotroph isolates (Dbs 1, Dbs 2, and Dbm 3) was constructed based on 16S rDNA sequences and compared with reference strains. As shown in the figure 2a, Dbs 1 isolates had the same cluster with several strains of *Acinetobacter schindleri*. Dbs 1 isolate had an identical sequence (99.9%) with *A. schindleri* ZSR6. While Dbs 2 and Dbm 3 was identified a member of genus *Pseudomonas* (Figure 2b). Dbs 2 and Dbm 3 isolates had a relationship with *P. syncyanea* NBRC3757T and *P. moraviensis* F9-6 with similarity value 100%, and 99.9%, respectively.

Several previous studies have identified the Genus *Acinetobacter* and *Pseudomonas* can be used as PGPR agents because they have multi-activities for enhancing plant growth. *Acinetobacter schindleri* YNB103 is the PGPR bacteria which is able to increase physiological metabolism, photosynthetic, vitality, and root respiration to effectively increase cherry growth (*Cerasus avium*) (Wenjie *et al*., 2015). Diazotrophic bacteria *Acinetobacter baumanii* LRFN53 which isolated from wheat rhizosphere Lokwan varieties has been shown biological activities in phosphate solubilizing and siderophores production (Sachdev *et al*., 2010). *A. baumanii* PUCM1029 isolate from Rokhbaksh-Zamin *et al*. (2011) has the ability to produce IAA hormones, production of siderophores, ZnO solubilizing, and phosphate solubilizing. Differences in the ability of each bacteria are suspected due to differences in bacterial species, sampling sites, and rhizosphere soil types.

Based on our knowledge, this is the first report *Pseudomonas syncyanea* was exhibited plant growth-promoting properties, but *Pseudomonas moraviensis* has been extensively studied about its potency as a PGPR agent and effectiveness as a biofertilizer. Biofertilizer containing *Bacillus cereus* and *Pseudomonas*

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moraviensis inoculums with corn straw and rice husk additives can increase the height and weight of wheat plants by 18-30%, increase protein content, proline, and sugar levels, and increase antioxidant activity in wheat plants by 25-40% (Hassan and Bano, 2016). *Pseudomonas* GRC2 isolated from potatoes root is able to colonize at the roots causing increased growth, increased nodulation, reduction of root disease symptoms, and an increase of seedlings in pea plants because of their ability to produce siderophores, HCN, IAA, and antibiotics (Gupta *et al*., 2001). *Pseudomonas* BHUJY23 can increase rice production because it has the ability to produce IAA, phosphate solubilization, and inhibit the growth of pathogenic *Rhizoctonia solani* (Lavakush *et al*., 2012).

Table 3: Acidity of growth medium before and after incubation time.

Isolate code	pH	
	Initial	Final
control	7.0a	6.96a
Dbs 1	7.0a	5.65b
Dbs 2	7.0a	5.69b
Dbm 3	7.0a	6.58b

* Data were expressed as mean ± standard deviation of three replicates analyzed using Paired-Sample T-test at α = 0.05

* Average values followed by different notations indicate that a significant difference between pH before and after treatments

Table 2: Phenotype characteristic of selected PGPR isolate.

Characteristic	Dbs 1	Dbs 2	Dbm 3
Colony morphology and pigmentation	round, white transparent	Round, white-yellow	Round, white cream
Cell structure	Short rod	Rod	Short rod
Gram reaction	-	-	-
Endospore	-	-	-
Motility	-	+	+
Catalase	+	+	+
Oxidase	-	+	-
Acid from :			
Glucose	+	+	+
Lactose	+	+	+
Mannitol	+	+	+
Sucrose	+	+	+
Galactose	+	+	+
Starch hydrolysis	-	+	+
Nitrate reduction	+	+	+
Indole production	-	-	-
H_2S production	+	+	+
Urease production	-	-	-
Protease production	-	-	-
Citrate utilization	-	+	+
Voges Poskauer reaction	-	-	-
Methyl Red reduction	-	-	-

\+ : positive - : negative

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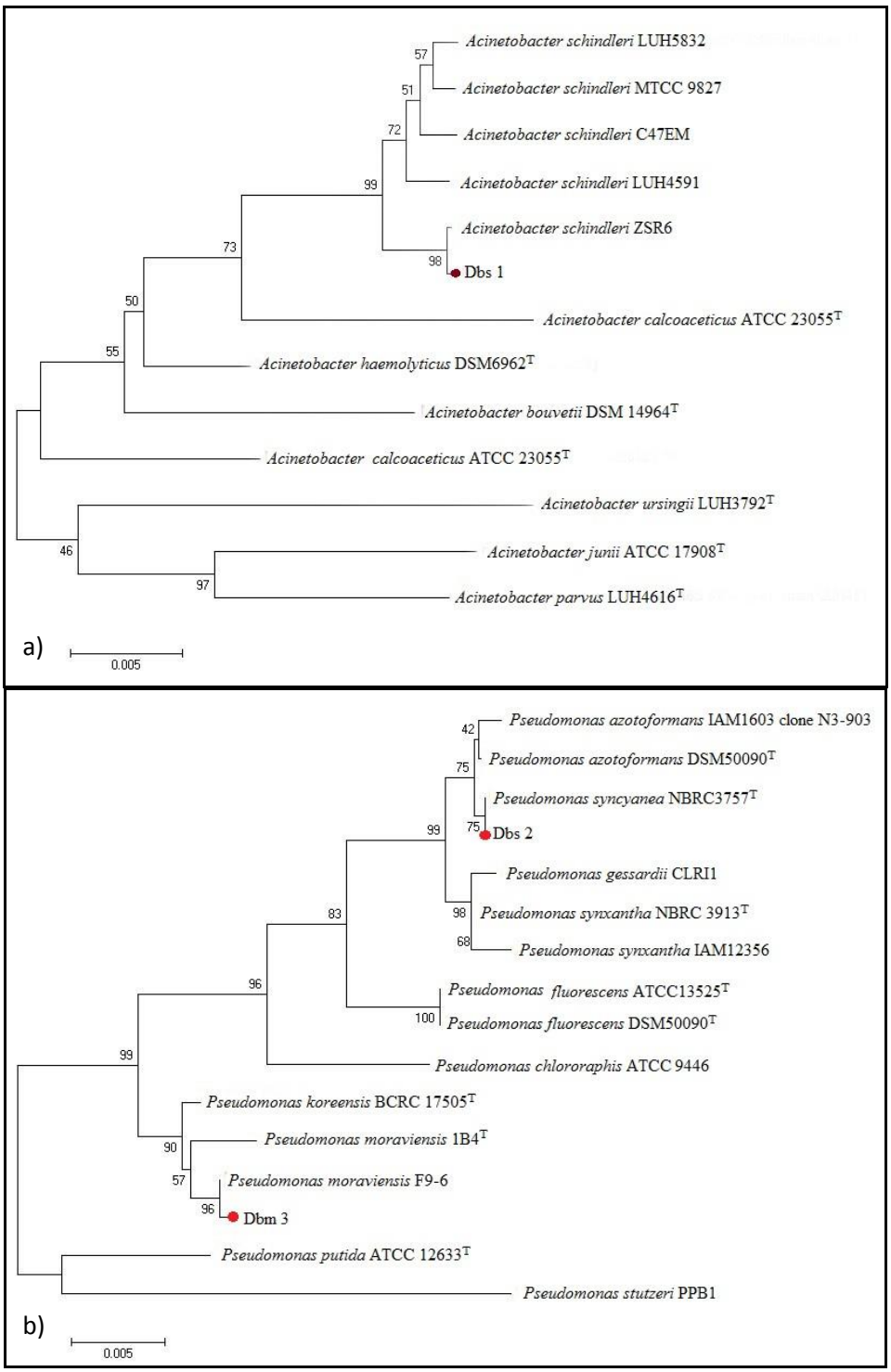

Figure 2: Phylogeny tree of diazotroph bacteria based on 16S rDNA sequence using Neighbor-joining algorithm a) Dbs 1 isolate b) Dbs 2 and Dbm 3 isolate.

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CONCLUSION

All of the candidate isolates from this study could be used as alternative PGPR. Further research is needed before the PGPR agents are ready to be applied on a commercial scale such as in vitro test to determine the population dynamics of PGPR agents during application, colonization tests to determine the ability of PGPR agents to colonize the rhizosphere, and to determine appropriate biofertilizer formulations to enhance the biofertilizer shelf-life.

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REFERENCES

Agbodjato, N. A., Noumavo, P. A., Baba-Moussa, F., Salami, H.A., Sina, H., Sezan, A. and Baba-Moussa, L. (2015). Characterization of potential plant growth promoting rhizobacteria isolated from maize (*Zea mays* L.) in central and northern Benin (West Africa). *Applied and Environmental Soil Science* **Vol 2015, Article ID 901656.**

Ahemad, M. and Khan, M. S. (2011). Assessment of plant growth promoting activities of rhizobacterium *Pseudomonas putida* under insecticide-stress. *Microbiological Journal* **1, 54-64.**

Ahmad, F., Ahmad, I. and Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research* **163, 173-181.**

Aziz, K., Nawaz, M., Nazir, J., Anjum, A. A., Yaqub, T., Ahmad and Khan, M. (2015). Isolation, characterization, and effect of auxin producing bacteria on growth of *Triticum aestivum*. *Journal of Animal and Plant Sciences* **25(4), 1003-1007.**

Baldani, J. I., Reis, V. M., Videira, S. S., Boddey, L. H. and Baldani, V. L. D. (2014). The art of isolating nitrogen-fixing bacteria from non-leguminous plant using N-free semi-solid media: A practical guide for microbiologists *Plant Soil* **384, 413-431.**

Cattelan, A. J., Hartel, P. G. and Fuhrmann, J. J. (1999). Screening of plant growth promoting rhizobacteria to promote early soybean growth. *Soil Science Society of America Journal* **63, 1670-1680.**

Chauhan, A., Balgir, P. P. and Shirkot, C. K. (2014). Characterization of *Aneurinibacillus aneurinilyticus* strain CKMV1 as a plant growth promoting rhizobacteria. *International Journal of Agriculture, Environment, and Biotechnology* **7, 37-45.**

Gupta, C. P., Dubey, R. C. and Maheswari, D. K. (2001). Plant growth enhancement and suppression of *Macrophomina phaseolina* causing charcoal rot of peanut by fluorescent *Pseudomonas*. *Biology and Fertility of Soils* **35, 399-405.**

Gupta, A., Rai, V., Bagdwal, N. and Goel, R. (2005). *In situ* characterization of mercury resistant growth promoting fluorescent pseudomonads. *Microbiological Research* **160, 385-388.**

Gupta, G., Panwar, J., Akhtar, M. S. and Jha, P.N. (2012). Endophytic nitrogen-fixing bacteria as biofertilizer. *In*: Sustainable Agriculture Reviews 11. Lichtfouse, E. (ed.), Springer, Dordrecht **pp. 183-221.**

Hanapi, S. Z., Awad, H. M., Sheikh Ali, S. I., Mat Sarip, S. H., Sarmidi, M. R. and Aziz, R. (2013). Agriculture wastes conversion for biofertilizer production using beneficial microorganisms for sustainable agriculture applications. *Malaysian Journal of Microbiology* **9(1), 60-67.**

Hassan, T. U. and A. Bano (2016). Biofertiler: A novel formulation for improving wheat growth, physiology, and yield. *Pakistan Journal of Botany* **48(6):2233–2241.**

Hoe, P. C. K., Rahim, K. A. and Norddin, L. (2015). Assessment of multifunctional biofertilizer on rice seedlings (MR 219) growth in a greenhouse trial. *Malaysian Journal of Microbiology* **11(2), 195-198.**

Islam, M. R., Sultana, T., Joe, M. M., Yim, W., Cho, J. C. and Sa, T. (2013). Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper. *Journal of Basic Microbiology* **53(12), 1004-1015.**

Iwata, K., Azlan, A., Yamakawa, H., and Omori, T. (2010). Ammonia accumulation in culture broth by the novel nitrogen-fixing bacterium *Lysobacter sp*. E4. *Journal of Bioscience and Bioengineering* **110(4), 415-418.**

Ji, S. H., Gururani, M. A. and Chun, S. (2014). Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiological Research* **169, 83-98.**

Khalid, A., Arshad, M. and Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology* **96, 473-480.**

Kumar, K. V., Singh, N., Behl, H. M. and Srivastava, S. (2008). Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. *Chemosphere* **72, 678-683.**

Kumar, V., Kayasth, M., Chaudhary V. and Gera, R. (2013). Diversity of diazotrophs in arid and semi-arid regions of haryana and evaluation of their plant growth promoting potential on Bt-cotton and pearl millet. *Annals Microbiology* **64 (3), 1301-1313.**

Lavakush, Yadav, J. and Verma, J. P. (2012). Isolation and characterization of effective plant growth promoting rhizobacteria from rice rhizosphere of Indian soil. *Asian Journal of Biological Sciences* **5, 294-303.**

Mihalache, G., Zamfirache, M., Mihasan, M., Ivanov, I., Stefan, M. and Raus, L. (2015). Phosphate-solubilizing bacteria associated with runner bean

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rhizosphere. *Archives of Biological Sciences. Belgrade* **67(3), 793-800.**

Prashant, D. S., Makarand R. R., Bhushan L. C., and Sudhir B. C. (2009). Siderophoregenic *Acinetobacter calcoaceticus* isolated from wheat rhizosphere with strong PGPR activity. *Malaysian Journal of Microbiology* **5(1), 6-12.**

Raut, V., Shaikh, I., Naphade, B., Prashar, K. and Adhapure, N. (2017). Plant growth promotion using microbial IAA producers in conjunction with Azolla: Novel approach. *Chemical and Biological Technologies in Agriculture* **4, 1-11.**

Rokhbaksh-Zamin, F., Sachdev, D., Kazemi-Pour, N., Engineer, A., Pardesi, K. R., Zinjarde, S., Dhakephalkar, P. K. and Chopade, B. A. (2011). Characterization of plant-growth-promoting traits of *Acinetobacter* species isolated from rhizosphere of *Pennisetum glaucum*. *Journal of Microbiology and Biotechnology* **21(6), 556-566.**

Sachdev, D. P., Chaundari, H. G., Kasture, V. M., Dhavale, D. D. and Chopade, B. A. (2009). Isolation and characterization of Indole Acetic Acid (IAA) producing *Klebsiella pneumoniae* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. *Indian Journal Experimental Biology* **47, 993-1000.**

Sachdev, D., Nema, P., Dhakephalkar, P., Zinjarde, S. and Chopade, B. (2010). Assessment of 16S rRNA gene-based phylogenetic diversity and romising plant growth-promoting traits of *Acinetobacter* community from the rhizosphere of wheat. *Microbiological Research* **165, 627-638.**

Saribay, G.F. (2003). Growth and nitrogen fixation Dynamics of *Azotobacter chroococcum* in nitrogen-free and OMW containing medium. Department of Food Engineering The Middle East Technical University, Turkey. M.Sc. Thesis.

Setiono, A. (2015). Inovasi Jeruk Keprok Batu 55. Balai Penelitian Tanaman Jeruk dan Buah Subtropika, Batu. **pp. 100-111.**

Sugiyatno, A. (2014). "Keprok Batu 55" Jeruk unggul nasional asal Jawa Timur. prosiding seminar nasional PERHORTI 2014. **pp. 149-56.**

Sugiyatno, A. (2015). Proses invensi menuju inovasi jeruk Keprok Batu 55. Balai penelitian tanaman jeruk dan buah subtropika, Batu. **pp. 91-99.**

Thakuria, D., Talukdar, N. C., Goswami, C., Hazarika, S., Boro, R. C. and Khan, M. R. (2004). Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. *Current Science* **86, 978-985.**

Walpola, B. C and Yoon, M. (2013). Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. *African Journal of Microbiological Research* **7(3), 266-275.**

Wang, Q., Xiong, D., Zhao, P., Yu, X., Tu, B. and Wang, G. (2011). Effect of applying an arsenic-resistant and plant growth-promoting rhizobacterium to enhance soil arsenic phytoremediation by *Populus deltoides* LH05-17. *Journal of Applied Microbiology* **111, 1065-1074.**

Wenjie, Z., Deguo, L., Dandan, Y. and Sijun, Q. (2015). Effect of plant growth-promoting rhizobacteria on photosynthesis and root vitality of sweet cherry saplings. *Journal of Jilin Agricultural University* **37(5), 555-567.**

Wilson, K. H., Blitchington, R. B. and Greene, R. C. (1990). Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *Journal of Clinical Microbiology* **28, 1942-1946.**

Wong, W., Tseng, C., Hsu, S., Lur, H., Mo, C., Huang, C., Hsu, S., Lee, K. and Liu, C. (2014). Promoting effects of a single *Rhodopseudomonas palustris* inoculant on plant growth by *Brassica rapa chinensis* under low fertilizer input. *Microbes and Environment* **29(3), 303-313.**

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