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SHORT COMMUNICATION

Characterization of an Endophytic Bacterium G062 Isolate with Beneficial Traits

ALINA AKHDIYA[1,2], ARIS TRI WAHYUDI[1*], ABDUL MUNIF[3], LATIFAH KOSIM DARUSMAN[4]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[2]ICABIOGRRD, Research & Development Institute, Ministry of Agriculture, Jalan Tentara Pelajar 3A, Cimanggu, Bogor 16111, Indonesia
[3]Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[4]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia

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An endophytic bacterium isolate G062 was characterized base on its molecular genetic potents, morphology, physiology, and biochemistry reactions. Analysis of 16S rDNA sequences of G062 showed the highest similarity to *Paracoccus halophilus* (98%). Detection of the *phlD* and *prnC* genes occurrence indicated that the bacterium had this antibiotic-like genes of Diacethylphloroglucinol (DAPG) and pyrrolnitrin. The cells are rod shaped (0.59-0.89 x 1.85-3.3 µm), aerobic, Gram negative, non motile, non spore forming, positive catalase, positive oxydase, could reduce NO_3 to N_2, nitrogen fixing, producing siderophore and plant growth hormones-like compounds (IAA, Gibberellin, and zeatin), and solubilizing phosphate. The G062 isolate could grow on media containing 2.5% NaCl. Range of the temperature and pH growth were 15-40 and 5.0-9.5 °C, respectively. The bacterium did not cause red blood cells lysis. There was no hypersensitive response when it was injected into tobacco leaves, and it was not pathogenic against potato plantlets. Moreover, the bacterium promoted the growth of the potato plant and had high colonization ability. These results suggested that the bacterium had beneficial and good traits as biological agent candidate to promote potato plant growth.

Key words: characterization, endophytic bacterium, *Paracoccus halophilus*, potato plant

INTRODUCTION

Endophytic bacteria are defined as bacteria that can be isolated from the plant tissue or from tissue that had been surface sterilized and does not harm to the plant (Hallmann *et al*. 1997). Today, the development of microbiology and biotechnology have prove that the presence of endophytic bacteria is essential for plant growth, plant resistance to biotic and abiotic stresses, and crop productivity. The roles of the endophytic bacteria are related to its abilities in nitrogen fixing, phytohormones producing, biocontrol of pathogens, as well as inducers of plant immunity (Mano & Morisaki 2008; Andreotte *et. al*. 2010).

Endophytic bacteria have great potential to be applied to seeds or plant seedlings as a way to increase/promote its growth and resistance; for its early application may protect their host plants from pathogen colonization. Research experiments showed that plantlets of potato, grape, and banana which were grown together with endophytic bacteria grown better and had higher resistancy against pathogens than the non-inoculated plantlets (Frommel *et al*. 1991; Krechel *et al*. 2002; Sessitsch *et al*. 2005; Faltin *et al*. 2004; Compant *et al*. 2005; Kavino *et al*. 2007).

Useful bacteria can be isolated from nature; furthermore its can be developed and used as artificial inocula to improve plant growth and resistance. Physiological and molecular chararacters of the bacteria are very important information which are needed as scientific basic to develop a good and safe bacterial inocula. Considering that reason, this research was conducted and addressed. The aims of this study was to characterize endophytic bacterium G062 isolated from potato stem plant.

*Corresponding author. Phone/Fax: +62-251-8622833, E-mail: aristri2011@gmail.com

MATERIALS AND METHODS

Bacteria and Preservation of the Culture. G062 isolate was an endophytic bacterium isolated from the surface of sterilized stem of potato plant (Atlantic) taken from Garut, West Java Indonesia (unpublished data). Working cultures was maintained routinely by growing on TSA (30 g/L TSB, 15 g/L agar) slant at room temperature (29-29 °C) and stored in refrigerator. The isolate was preserved in 40% glycerol at -20 °C and in lyophilized form for long-term storage.

Pathogenicity Potent Test of the Isolate. The isolate was targeted to haemolytic and plant pathogenicity tests. Haemolytic activity of the isolate were evaluated by streaking G062 on blood agar. Plant pathogenicity tests were conducted against non host plant by Hipersensensitive Respon (HR) bioassay on tobacco leaves (*N. tobaccum*) and host plantlets (*S. tuberosum*) in 5 replicates. *E. coli* (as negative control) was grown by streaking in LA (5 g yeast extract, 10 g trypton, 10 g NaCl, and 15 g agar) for 2 days, while *R. solanacearum* (as positive control) and G062 bacterium isolate were grown by streaking in TSA for 5 days. The bacterial colonies were scrabbed using loop inoculation. The bacterial cells were suspended in 1 mL of physiological saline solution (± 10^7 CFU/mL). Each suspension was injected into tobacco leaves (*Nicotiana tabaccum*) according to the method described by Zou *et al*. (2006). Tobacco leaves were observed for 7 days to determine the hypersensitive response of the infected tissue around the infected site. A total of 100 µL bacterial suspension (± 10^7 CFU/mL in distilled water) were dropped onto the medium around the plantlet root in culture tubes containing the 2-weeks old plantlets. The effect of bacterial inoculation on plantlets was observed for 10 days.

Molecular Characterization. The genomic DNA were extracted using *XPrep Stool DNA Mini Kit* (Philekorea Technology, INC. Seoul Korea). Molecular identification of bacteria were done based on sequence analysis of 16S rRNA gene. The 16S rRNA gene amplification used 63f (5'-CAGGCCTAACACATGCAAGTC-3') and 1387r (5'-GGGCGGWGTGTACAAGGC-3') primers and PCR condition as described by Marchessi *et al*. (1998). A pair of IGK (TACGGYAARGCBGGYATCGG) and NDR-1 (TTGGAGCCGGCRTANGCRCA) primers published by Poly *et al.* (2001) and Valdes *et al*. (2005) were used as forward and reverse primers, respectively, for amplification of the *nif* gene. PCR reactions of *nif* gene were conducted in 30 cycles (2 min initial denaturation at 95 °C, 1 min denaturation at 94 °C, 2 min annealing at 56.5 or 57 °C, 2 min elongation at 72 °C, and 7 min post elongation at 72 °C). Genetic potent of 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrine production were examined by using pairs of *phl2a* (5'-GAGGACGTCGAAGACCACCA-3') and *phl2b* (5'-ACCGCAGCATCGTGTATGAG-3'), *phen*1 (5'-CCCCTGTTGACAATTAATC-ATCGG-3') and *phen*2 (5'-ACCTTGACGTTGTACCATTCCCAA-3'), and *prn*Cf (5'-CCACAAGCCCGGCCAGGAGC-3') and *prn*Cr (5'-GAGAAGAGCGGGTCGAT GAAGCC-3') primers, respectively. Amplification condition of the two metabolite genes conducted as published by Raaijmakers *et al*. (1997) and Mavrodi *et al*. (2001), respectively. CLUSTAL W program (Thompson *et al.* 1994) was used for performing the 16S gene sequences alignment. Construction of the phylogenetic tree was made by using the neighbour-joining method with Maximum Composite Likelihood model in Mega 5.05 (Tamura *et al*. 2011). To confirm the reduction activity of NO_3 to N_2, occurrence of the *nos* gene was tested by PCR using a pair of *nos*661F (5'-CGGCTGGGGGCTGACCAA-3') and *nos*1773R primers (5'-ATRTCGATCARCTGBTCGTT-3') as stated by Scala and Kerkhof (1988). PCR conditions of *nos* gene were conducted in 30 cycles (2 min initial denaturation at 95 °C, 1 min denaturation at 94 °C, 2 min annealing at 55 °C, 2 min elongation at 72 °C, and 7 min post elongation at 72 °C).

Morphological Observation. Colony morphologies (color, shape, elevation, and consistency of the colony) were examined on TSA and KBA (20 g/L protease pepton, 1.5 g/L $K_2HPO_4{\cdot}3H_2O$, 1.5 g/L $MgSO_4{\cdot}7H_2O$. 20 ml/L glycerol, 15 g/L agar). Morphology and size of log phase cells (from 24 h TSB culture) were observed using Scanning Electron Microscope (Jeol JSM-5310 LV) at 10,000 magnification. The specimens were fixed in cacodylate buffer and glutaraldehyde, dehydrated in alcohol, and then freeze dried. Before observed, the specimen were cut, attached to stub specimens with double cello-tape, coated with gold 400 A◦ in Eico I-B2 ion coater (Goldstein 1992). Motility of the cells were examined by observation of hanging drop preparation slides under bright field microscope (Olympus CH20BIMF200).

Physiological and Biochemical Characterization. All of the cultures used for the tests were incubated at room temperature (29-30 °C) unless otherwise stated. Nitrogen fixation ability was tested by growing on Nitrogen Free Agar (NFA) and LGI Agar which was modified (0.2 g K_2HPO_4; 0.6 g KH_2PO_4; 0.2 g $MgSO_4{\cdot}7H_2O$; 0.02 g $CaCl_2$; 0.002 g $Na_2MoO_4{\cdot}2H_2O$; 0.01 g $FeCl_3$; 3 g arabinose; 3 g manitol; 3 g malic

acid; and 15 g agar). Acetylene reduction activity test was conducted toward modified the LGI semisolid culture in triplicate.

Capability of G062 to produce plant growth hormones-like were determined in TSB. A loop full of colony which were grown on TSA for 48 h were inoculated into the test tube containing 5 mL TSB, then the tube was incubated on incubator by shaking (100 rpm) at room temperature (29-30 ^{o}C). After 24 h, 1 mL of the liquid culture was inoculated into a flask containing 50 mL new sterile TSB. The flask was incubated at room temperature (29-30 ^{o}C). After 7 days, the culture was centrifuged at 4 ^{o}C, 10,000 rpm, for 10 min. Quantification of IAA-like in the supernatant cultures were conducted in 4 replicates by using the Salkowsky methods which has been modified by Glickmann and Dessaux (1995). Gibberellin (GA)-like, and zeatin-like contained in the supernatant, were extracted and determined triplicates by using the methods described by Ergűn *et al*. (2002).

The Gram reaction of the cells grown on TSA (24 h) were examined by using non staining (Buck 1982) and staining techniques (Benson 2001) under bright field microscope (Olympus CH20BIMF200) at 1000x magnification. Chitinolytic, proteolytic, and amylolytic activities were observed to the culture that has grown for 7 days on 1% (w/v) TSA containing 1% colloidal chitin, 1% skim milk, and 1% soluble starch, respectively. Hydrolysis of CMC (Carboxy methyl cellulose) was observed by streaking of isolate on CMC agar. Capability of the isolate to produce siderophore was tested according to the method described by Hu and Xu (2011). Phosphate solubilization activity was observed on Pikovskaya agar plate (Pikovskaya 1948). Catalase activity was tested by bubbles formation on 3% H_2O_2 solution that was mixed with a loop of bacterial colony grown for 24 h on TSA. Oxidase activity was determined by using oxidase paper (Difco). NaCl tolerance was tested using LB medium contained appropriate concentration of NaCl. The bacterium were inoculated into TSB or adjusted TSB (by using NaOH and HCl) and then incubated at various temperature (13, 18, 20, 30, 37, 40, and 42 ^{o}C) or room temperature (30 ± 1 ^{o}C) to determine its tolerance against temperature and pH of growth. Growth were checked by measuring optical density (600 nm) of the cultures. Other physiological and biochemical reactions were examined by applying API 20NE galleries (bioMerieux SA, Lyon, France).

Evaluation of Colonization Ability. Colonization of G062 isolate on internal stem tissue of potato plantlet was observed by using Scanning Electron Microscope (Jeol JSM-5310 LV). The specimens were prepared according to Goldstein (1992) as we described above before observed at 750x and 1000x magnification. Re-isolation of G062 isolate was also conducted to evaluate the colonization ability of G062 isolate inside the plantlet tissue. The shoot of the plantlets were cut at 1.5 cm above the surface media, weighed, and grinded. The plantlet extract were diluted and plated on 50% TSA.

Observation of Plant Growth Promotion Ability. Potato plantlets (2 weeks after subcultured) were inoculated with G062 suspension as described above. Two weeks after inoculation, the G062 inocula and control plantlets were planted on 3 kg sterile planting media consisted of Chicken's manure, soil, and risk husk (ratio = 1:1:1). Planting was conducted at Research station of ICABIOGRRD, in Pacet, District of Cianjur, West Java, Indonesia. Three months after planting, the plant biomass (plant shoot and root) were harvested, dried, and weighed; while the tuber were calculated and weighed. Growth parameter and tuber productivity value were calculated from 10 plants.

RESULTS

Pathogenicity Test of G062 Isolate. There were no hydrolytic zone showed on Blood agar (data not shown), nor necrotic tissue around the infected sites of tobacco leaves during the observation (7 days after streaking or injection of G062 cells suspension). Pathogenicity test on potato plantlet also showed no disease symptoms or plantlets killed by the bacterium; moreover, all the plantlets looked healthy during observation as shown in Figure 1.

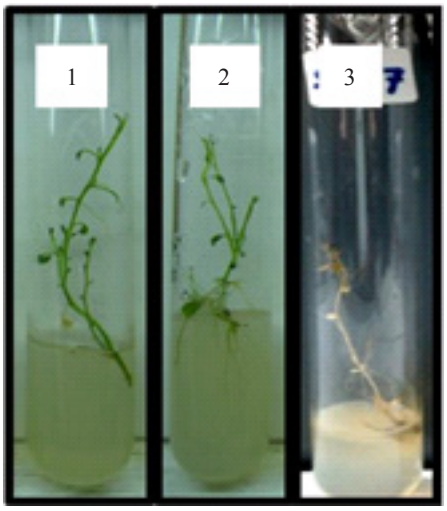

Figure 1. The effect of bacterial inoculation on potato plantlets. 1. Healthy plantlet after G062 inoculation, 2. Healthy plantlet without bacterium inoculation, 3. Plantlet killed after phytopathogenic bacterium inoculation.

Molecular Characters of G062 Isolate. Sequence analysis of 16S rRNA gene showed that G062 isolate was belong to genus *Paracoccus*, one of an α proteobacteria group. The closest related species was *Paracoccus halophilus* (98%, max. score 1245, query cover 100%, e value 0.0, accession No. NR. 043810.1). *P. versutus* (98.0%, max. score 1229, query cover 100%), *P. pantotrophus* (98%, max. score 1223, query cover 100%), *P. marinus* (98%, max. score 1221, query cover 98%), and *P. koreensis* (98%, max. score 1216, query cover 98%) were the next closely related ones. Sequences alignment of phylogenetic trees were constructed with the neighbour-joining, maximum-parsimony and maximum-likelihood methods. Values of the bootstrap were evaluated by using 1000 replication. The phylogenetic tree placed the isolate in a coherent cluster with *P. halophilus* and closer relation to *P. koreensis* (Figure 2). Detection of the *nif*HD, *nos, prn*C and *phl*D gene occurrence by PCR yielded multiband DNA amplicons (Figure 3 & 4). The *nif* genes amplicons were approximately 1200, 1400, and 1700 bp in size. Two sharp DNA bands (± 450 and 550 bp) and 4 faint bands (± 850, 1110, 1300, and 1500 kb) were detected on electrophoresis gel of *nos* gene amplicons. Two faint bands (both were smaller than 500 bp), and two clear bands (± 720 and 1300 bp) were detected on PCR product of *prn*C gene; and 5 PCR products (3 clear bands: ± 1200, 500, 300 bp; and 2 faint bands : ± 550 and 265 bp) were obtained by using the *phl*D primers.

Description of Morphology and Cytological Characters. Colonies of G06 isolate were round, smooth, translucent (on KBA) or cream to faint brown (on TSA), and had soft consistency. The colonies on modified LGI agar are small (2-5 mm

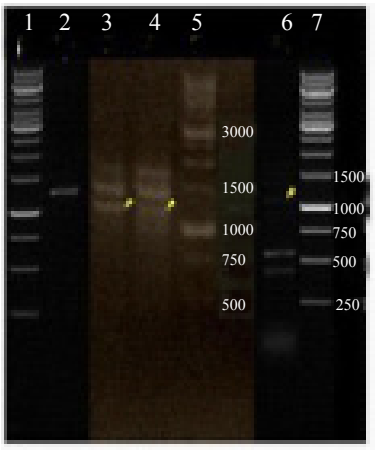

Figure 3. PCR products of 16SrDNA, nitrogen fixation and nitrate reduction genes of G062 isolate. The arrowheads indicated the expected DNA amplicon of each genes. 1. 1 kb ladder (Geneaid), 2. 16S rDNA, 3. *nif* HD gene (56.5 °C), 4. *nif* HD gene (57 °C), 5. 1 kb ladder (Thermo), 6. *nos* gen, 7. 1 kb (Geneaid).

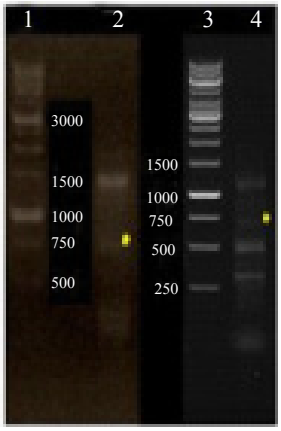

Figure 4. Amplification products of antifungal compounds genes of G062 isolate. The arrowheads indicated the expected DNA amplicons of each genes. 1. 1 kb ladder (Thermo), 2. *prn*C gene, 3. 1 kb ladder (Geneaid). 4. *phl*D gene.

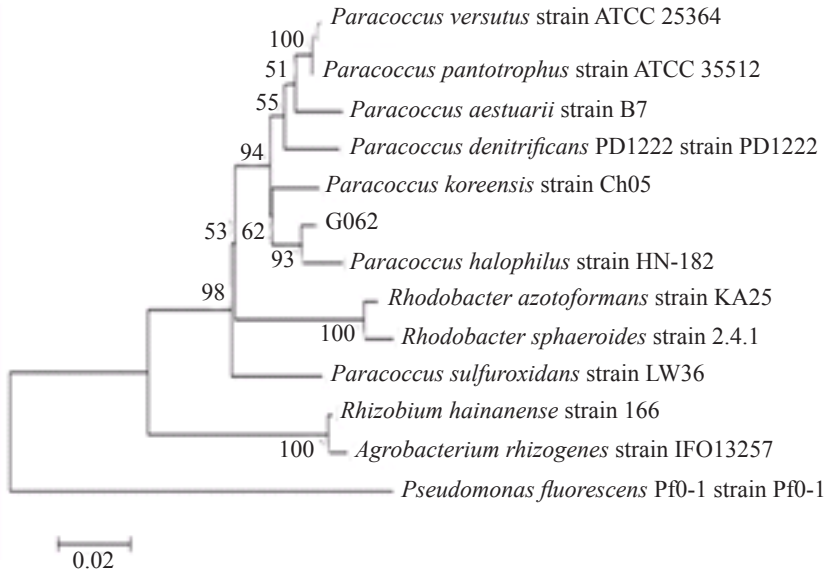

Figure 2. Phylogenetic tree of G062 isolate based on 16S rDNA sequence compared with the reference strains.

in 10 days), watery, and translucent or opaque. There were no pellicle on liquid cultures (on TSB or modified LGI). Shape of the exponential phase cells grown on TSB were short rod or rod. The cells were non motile and there were no endospore formation observed. Dimension of the exponential phase of single cells grown in TSB were 0.59-0.89 x 1.85-3.3 µm. There were a lot of fibrous material around and covered the cells as shown in the electron photomicrograph (Figure 5).

Description of Physiological and Biochemical Characters. Cells of G062 isolate are Gram-negative, aerobic, and showed positive of catalase and oxidase activity. Growth observed at 15-40 °C but not at 13 or 43 °C. Cells growth were detected at pH 5.0-9.5 but not at pH 4.5 or 10.0. Growth of the liquid cultures (on TSB) were observed on TSB contained 2.5% (w/v) NaCl, but there were no increasing optical density at 5% NaCl. This isolate could completely reduced nitrate to dinitrogen. Indol production, urease, arginin dihydrolase, and hydrolysis of aesculin, gelatin, starch, chitin, skim milk, and CMC were negative. It also capable of utilizing and fermenting D-glucose, assimilating L-arabinose, mannose, mannitol, malic acid, D-fructose, lactose, and glycerol as its Carbon sources. On the other hand, N-acetyl glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, phenyl acetic acid, D-galactose, maltose, sucrose, and salicine were not assimilated (Table 1). The isolate also showed phosphate solubilization activity on Pikovskaya agar (Figure 6) and siderophore

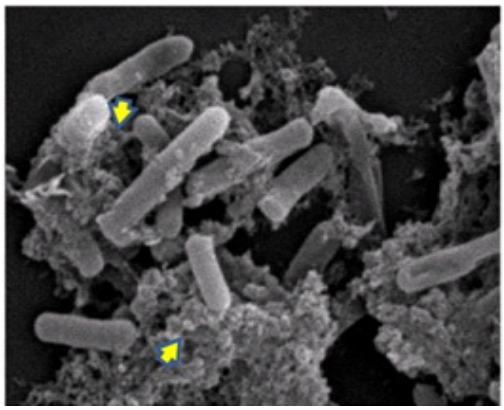

Figure 5. Scanning electron micrograph of G062 cells (at 10,000 magnification) which were grown on TSB for 24 hours. Fibrous materials around the cells were produced as indicated by arrowhead.

Table 1. Characteristics of closely related *Paracoccus* species and G062 strain

Characteristics	G062	*P. halophilus* HN-182T (Liu *et al*. 2008)	*P. versutus* (Katayama *et al*. 1995)	*P. koreensis* (La *et al.* 2005)	*P. denitrificans* (Nokhal & Schlegel 1983)
Motility	-	-	+	-	-
Size of cells (µm):					
width	0.59-0.89	0.4-0.5	0.4-0.5	0.5-1.0	0.6-0.85
length	1.85-3.3	0.7-1.1	1.1-1.7	1.0-1.5	0.8-1.2
Urease	-	+	NA	-	-
C source utilization:					
D-glucose	+	+	+	-	+
L-Arabinose	+	+	+	-	+a
Gluconate	-	NA	+	-	+
D-sorbitol	-	+	NA	+	+
Fructose	+	+	+	-	+
Galactose	-	+	+	-	+
Maltose	-	+	+	-	+
Sucrose	-	-	+	-	+
Mannose	+	+	+	-	+
Trehalose	-	-	NA	-	+
Mannitol	+	+	+	-	+
Citrate	-	NA	V	-	-a†
Lactose	+	-	-	-	-
Glycerol	+	+	+	-	+
Salicin	-	NA	NA	-	NT
Nitrate to N_2	+	-	+	-c	+
Growth at:					
Initial pH	5.0-9.5	5.0-9.0	6.5-9.5	6-8	6.0-10
Temperature (°C)	15-40	7-42	17-40	15-37	10-40
Max. of NaCl (%)	2.5	8.5	NA	NA	3-7b

a: most of the strains; a†: most of the strains (except mutant); b: depend on the strains; c: could not reduce NO_3, but could reduce NO_2; V: various reaction were reported; NA: data not available.

production on simple-double layer Chromo azurol agar.

The result of ARA bioassay showed that G062 isolate could fixed nitrogen from the air. Beside its ability to fix nitrogen, G062 isolate could produce plant growth hormone-like substances (IAA, Giberellin, and Zeatin-like), even though it was grown in media which was not supplemented with tryptophan. The bioassay result of ARA and measurement of plant growth hormone produced by G062 isolated are show in Table 2.

Micrographs of scanning electron microscope showed occurence of G062 colonies inside stem tissue of the potato plantlet (Figure 7). The microscopic observation were in line with the re-isolation result.

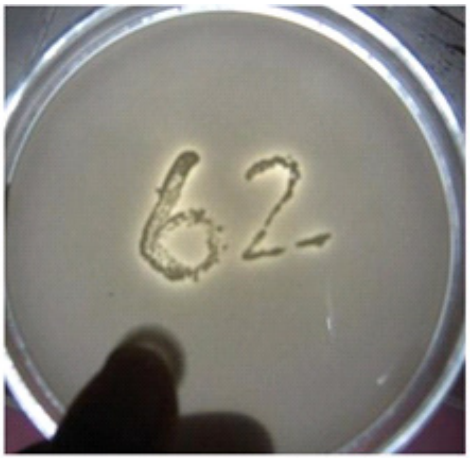

Figure 6. Phosphate solubilizing activivity of G062 isolate on Pikovskaya agar incubated at 29-30 ^{o}C for 72 hours.

Table 2. Nitrogen fixation activity on modified semisolid LGI and phytohormones-like compounds produced by G062 isolate in TSB medium

Assay	Results
ARA (µmol/mL/h)	1.190 ± 0.011
IAA-like (ppm)	17.4 ± 0.03
Gibberellin-like (ppm)	208 ± 7.16
Zeatin-like (ppm)	6.06 ± 0.23

Four weeks after inoculation, the average of G062 cell density on plantlets tissue was 2.8 x 10^4 ± 4.9 x 10^3 cfu/g. In comparison to control plants, the G062 inoculated plants produced higher plant biomass. Moreover, G062 plants produced in average 6.8 tuber/plants, while control plants produced only 5.1 tuber/ plants.

DISCUSSION

G062 isolate is not potent as mammalian pathogen, because it did not formed greenish or clear zone under and around the bacterial colonies which mean it did not lyse the red blood cells contained in the blood agar medium. Moreover, hypersensitive response (HR) bioassay result indicated that the isolate is not a phytopathogenic bacterium, because there were no nectrotic tissue observed at and around the infected sites of the tobacco leaves. This bioassay is a common tool to screen phytopathogenic bacteria. HR is a plant mechanism to prevent the recognized phytopathogenic bacteria growth and spreading from its entry sites. Heath (2000) stated that the mechanism involves a complex form of programmed cell death (PCD) which is consistently associated with the induction of local and systemic defence responses, and it can be the result of multiple signaling pathways. All of potato plantlets which were inoculated with the bacterium were also showed healthy condition and there were no disease symptoms appeared during observation time. According to the pathogenicity test results, it will be safe to be developed as bacterial inocula.

Base on partial 16S rDNA sequences, G062 isolate is a member of the metabolically versatile genus *Paracoccus*. Since the genus was created in 1969, at least 30 species were published as a part of it (Kelly *et al*. 2006; Roh *et al.* 2009; Deng *et al.* 2011; Lee *et al.* 2011; Zheng *et al.* 2011). Most

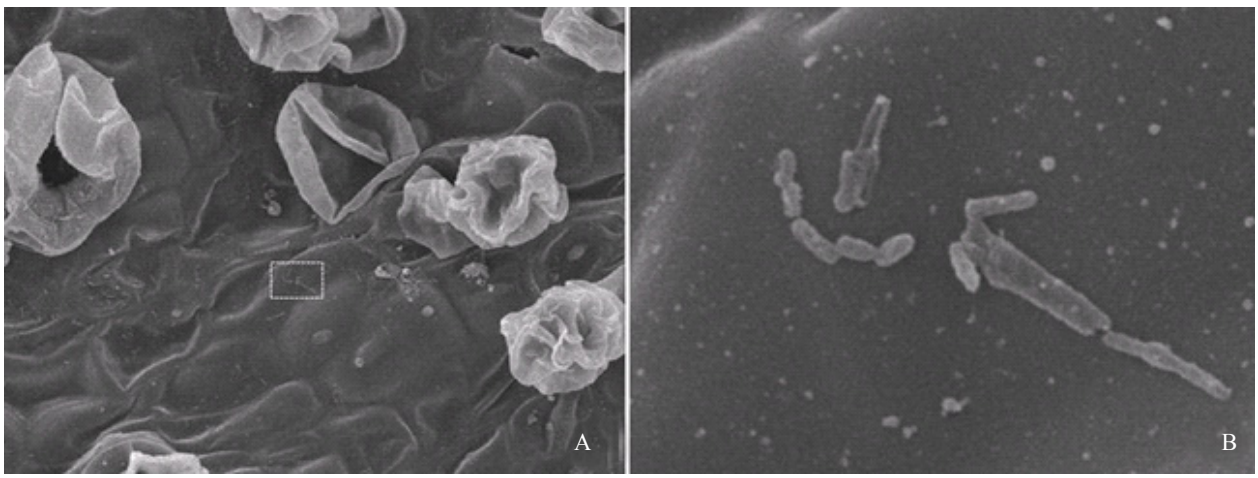

Figure 7. Colonies of G053 inside plantlet stem at 750x (A) and 10000x magnification (B).

of its were isolated from soil, marine, sediment or sludges. Recently, the well characterized (molecular, physiological, and biochemical) of *Paracoccus* species that has been reported as endophytic isolate was only *P. sphaerophysae* Zy-3(T) (Deng *et al.* 2011). However, the finding that the G062 isolate was a *Paracoccus* was not surprising, because some publication on plant bacterial diversity or community studies indicated the occurrence of genus *Paracoccus* in plant such as banana cv. Grand Naine shoot tips (Thomas & Soly 2009), *P. vulagaris* seedlings (Lopez *et al*. 2010), eucalyptus seedlings (Ferreira *et al*. 2008), and mangrove roots (Flores-Mireles *et al*. 2007). Surprisingly, G062 isolate showed highest similarity to *Paracoccus halophilus* strain HN-182 which was isolated from marine sediment of South China sea (Liu *et al*. 2008). The next closest related species were *P. versutus* and *P. pantotrophus*. The BLASTN alignment result showed slight different with the phylogenetic tree that the isolate was in a coherent cluster with *P. halophilus* and closer relation to *P. koreensis* than *P. panthotrophus* and *P. versutus* (Figure 1).

Micrograph of scanning electron microscope showed fibrous material around and covered the cells (Figure 5). Nokhal and Schlegel (1983) also have reported the finding of fibrous material which covered *P. denitificans* cells. They stated that it has indication as extracellular polyanions and acidic mucosubtances, for it has reacted with Ruthenium red.

Compared with published closely related species (Table 2), *P. halophilus* G062 could tolerate higher pH but lower NaCl concentration (2.5%) than *P. halophilus* HN-182^T. Its lower NaCl tolerance may correspond to the condition of the internal part of plant as its natural ecological niche. Moreover, G062 was like *P. panthotrophus, P. versutus,* and *P. denitrificans* because they could reduced nitrate to dinitrogen, while *P. halophilus* HN-182^T could not. The activity of Nitrate reduction to N_2 and ARA bioassay were in line with the result of PCR product of *nos* and *nif*HD genes, respectively. The *nos* gene encodes nitrous oxide reductase which is needed to convert N_2O to N_2 at the final step of denitrification pathway (Jones *et al*. 2008), while *nif*HD encodes Fe-protein and α subunit of Mo-Fe protein of dinitrogenase reductase. We have expected that the size of the PCR product will be ± 1112 and 1200 bp for *nos* and *nif*HD genes, respectively. But, both of the PCR products showed multiple DNA bands (Figure 3) that indicated unspecific amplification which may caused by less specificity of the primers or non optimum PCR condition. The primer set to amplify *nos* gene (*nos*661F and *nos*1773R) were designed base on *nos* gene sequences of *P. stutzeri* Zobell (Scala & Kerkhof 1988). NDR-1 and IGK were universal primer for *nif*HD gene (Poly *et al*. 2001; Valdez *et al*. 2005), and often use at the first step of nested PCR of *nif*HD genes. Moreover, *Paracoccus* is a member of α-proteobacteria, which among it's group member shared conserved inserts and deletions (indels) in their molecular sequences (Gupta 2005) that may contributed to this unspesific *in vitro* amplification.

A diverse type of secondary compounds have been discovered as the product of endophytic microorganism. PCR of secondary metabolites encoding genes were conducted by using primers, *prn*Cf/*prn*Cr and *phl*2a/*phl*2b designed base on the *Pseudomonas fluorescen* genes that we expected to amplify a 719 and 745 bp DNA region of *prn*C and *phl*D, respectively. The *prn*Cf and *prn*Cr primers were amplified the *prn*C, one of four genes which involved in pyrrolnitrin biosynthesis (Kirner *et al*. 1998). While, the *phl*2a and *phl*2b primers were amplified the *phl*D, one of six genes which involved in the biosynthesis of a secondary metabolite 2,4-diacetylphloroglucinol (Raaijmakers *et al*. 1997). These substances has been known as broad spectrum antifungal antibiotic. Although PCR product *prn*C and *phl*D genes also showed multibands DNA, its indicated that *P. halophilus* G062 may has potency to produce pyrrolnitrin and 2,4-diacetylphloroglucinol (DAPG) compound. Beside, its may contribute to the non specific amplifications as we have stated before; occurrence of indels in region of the target gene may also contribute to the difference of DNA amplicons size of *phl*D. Insertion or deletion will change the gene length, and may affect its protein function. In addition to these antibiotic-like genes, *P. halophilus* G062 could produced siderophore which is also act as antifungal.

Phosphorus place is in the second position as most commonly limiting mineral factor for growth of terrestrial plants. Ironically, large of total P may reserve in soils, but it availability for plants is usually in a small proportion. Its low availability is because majority of its form found in insoluble forms (Verma *et al*. 2010). *P. halophilus* G062 formed the halo zone around its colonies on Pikovskaya agar (Figure 5). The halo zone indicated that there are tricalcium phosphate solubilizing activity into soluble form due to organic acids excreted by the bacterial cells. Its ability to solubilize tricalcium phosphate *in vitro*, may be applied as bacterial inocula to increase phosphate availability for plants.

Production of single phytohormones such by microorganisms has been reviewed by various scientists over the last 20 years (Verma *et al*. 2010). IAA, GA, and zeatin was detected on liquid cultures of *P. halophilus* G062 (Table 2). The ability of *P. halophilus* G062 to produce the phytohormone-like compounds is a beneficial character, because phytohormones analogous produced by endophytic bacteria can promote plant growth. Auxin are responsible for division, extension, and differentiation of plant cells and tissue. Stimulation of seed and tuber germination; increasing of xylem and root formation rate; controlling of vegetative growth, tropism, florescence, and fructification processes; affecting of photosynthesis, pigmen formation, metabolites biosynthesis, and plant resistance against stress are correspond with auxin (Tsavkelova *et al*. 2006). GA are a class of phytohormones which most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Verma *et al*. 2010). Zeatin is one of adenine type cytokinin which regulates cell growth, cell differentiation, apical dominance, and leaf senescence (Xu *et al*. 2012).

Naturally, endophytes and some phytopathogens live in same ecological niche, so colonizing and dominating capacity of the endophythes are important to its host plants. Hallmann and Bergh (2006) had reviewed that average density of natural endophytic bacteria in various plants root or stem were 10^4-10^6 cfu/g. This experiment showed that 2 weeks after inoculation of *Paracoccus halophilus* G062, cell density of the bacterium on the plantlets was 2.8 x 10^4 ± 4.9 x 10^3 cfu/g. Microscopic observation results also showed that the bacterium was found at internal tissue (sponge parenchyme) of the plantlet (Figure 7). The result indicated that the bacterium has high colonization ability. High colonization ability and persistency are important characters of a good biological agent candidate. The results also supported and prooved that the bacterium is an endophyte, because beside it was isolated from surface sterilized plant as stated in the materials and methods, the bacterium also showed to live or have life cycle inside the deep area of the plant tissue without harming the host plant, as well as endophytic defined previously by Wilson (1995).

Plant growth (dry weight of the potato shoot and root) and tuber productivity of G062 inoculated plant were higher than control plants (Figure 8). G062 plants looked vigour and dark green, while the control plants looked dwarf and light green to yellowish. This result obtained from 3 planting were consistent (data from preliminary G0 and next G1 plants parameter observation were not shown). It was suggested that interaction between the bacterium with the host plant significantly promoted the plant growth. Capability of *Paracoccus halophilus* G062

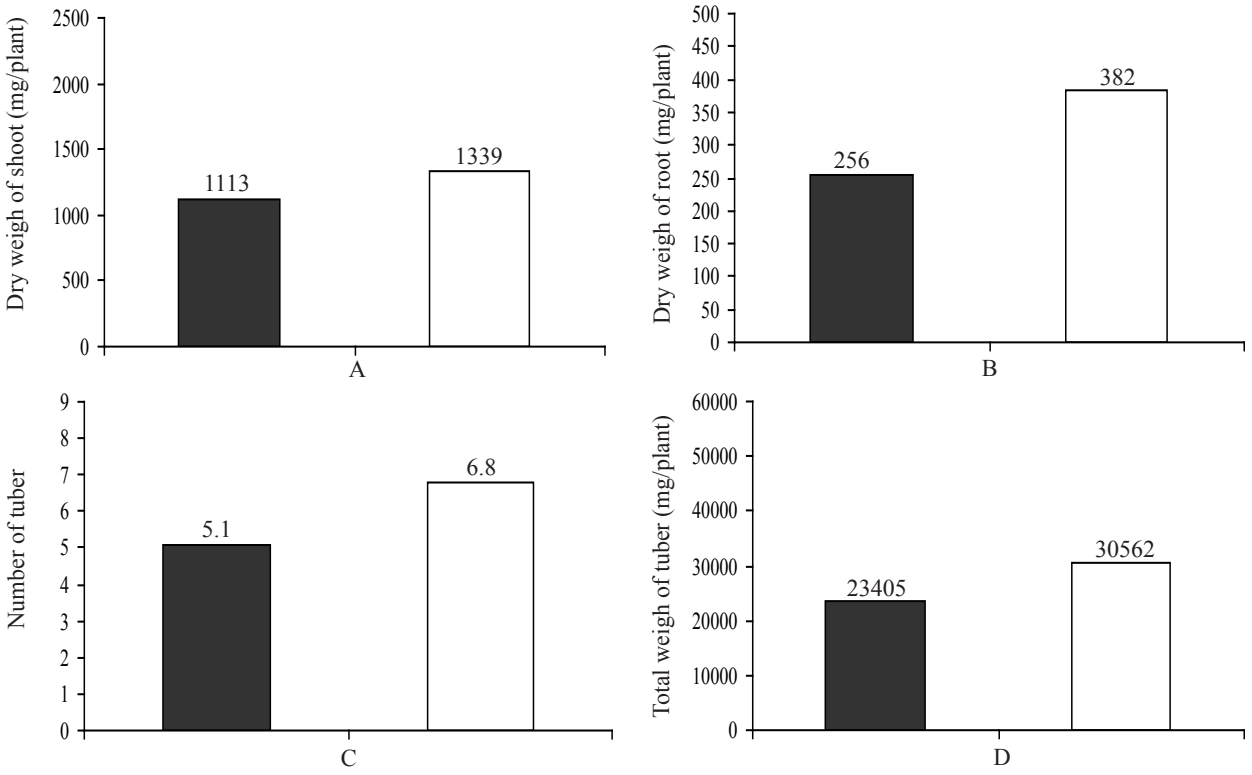

Figure 8. Growth parameters and tuber productivity of non enriched (■) and G062 enriched potato plants (□).

to fix Nitrogen, to produce plant growth hormones, and to solubilize the insoluble phosphate are beneficial characters that contribute to increase the nutrient availability to the plant. Nitrogen is a important element for plant growth. This element is a component of protein, nucleotides, and chlorophyls, three important molecules of plant cells. Compare to the control plants, it seem that nitrogen requirement of G062 plants were supplied sufficiently than control plants. Its strongly indicated that *P. halophilus* G062 contributes to supply the nitrogen requirement of the host. In addition, siderophore production was valuable weapon that may enhance its capacity to combat and compete with phytopathogenic fungi. Therefore, it may dominate inner part of plant tissue and control the pathogens. Beside its antifungal activity, siderophore increase plant capability to absorb Fe (Robin *et al*. 2006). For all of its beneficial characters, the bacterium is safe and has great potency to be developed as a component of artificial plant growth promoting (PGP) inocula.

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