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Paenibacillus yonginensis DCY84^T induces changes in *Arabidopsis thaliana* gene expression against aluminum, drought, and salt stress

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ABSTRACT

Current agricultural production methods, for example the improper use of chemical fertilizers and pesticides, create many health and environmental problems. Use of plant growth-promoting bacteria (PGPB) for agricultural benefits is increasing worldwide and also appears to be a trend for the future. There is possibility to develop microbial inoculants for use in agricultural biotechnology, based on these beneficial plant–microbe interactions. For this study, ten bacterial strains were isolated from Yongin forest soil for which *in vitro* plant-growth promoting trait screenings, such as indole acetic acid (IAA) production, a phosphate solubilization test, and a siderophore production test were used to select two PGPB candidates. *Arabidopsis thaliana* plants were inoculated with *Paenibacillus yonginensis* DCY84^T and *Micrococcus yunnanensis* PGPB7. Salt stress, drought stress and heavy metal (aluminum) stress challenges indicated that *P. yonginensis* DCY84^T-inoculated plants were more resistant than control plants. *AtRSA1*, *AtVQ9* and *AtWRKY8* were used as the salinity responsive genes. The *AtERD15*, *AtRAB18*, and *AtLT178* were selected to check *A. thaliana* responses to drought stress. Aluminum stress response was checked using *AtAIP*, *AtALS3* and *AtALMT1*. The qRT-PCR results indicated that *P. yonginensis* DCY84^T can promote plant tolerance against salt, drought, and aluminum stress. *P. yonginensis* DCY84^T also showed positive results during *in vitro* compatibility testing and virulence assay against *X. oryzae* pv. *oryzae* Philippine race 6 (PXO99). Better germination rates and growth parameters were also recorded for the *P. yonginensis* DCY84^T Chuchung cultivar rice seed which was grown on coastal soil collected from Suncheon. Based on these results, *P. yonginensis* DCY84^T can be used as a promising PGPB isolate for crop improvement.

1. Introduction

Plant growth-promoting bacteria (PGPB) include species which form specific symbiotic relationships with plants (*e.g.*, *Rhizobia* spp. and *Frankia* spp.), those which are free-living, bacterial endophytes which can colonize some or a portion of a plant's interior tissues, and cyanobacteria (Bashan et al. 2004). PGPB are bacterial strains isolated from diverse environments which are able to beneficially affect many parameters of plant growth and yield, directly or indirectly (Diaz-Zorita and Fernandez-Canigia 2009). Plant growth is directly promoted by PGPB either by facilitating nutrition uptake or by modulating the plant hormone levels. Indirect promotion of plant growth occurs *via* PGPBs decreasing the inhibitory effects of various pathogenic agents on plant growth and development (*i.e.*, acting as a biocontrol agent). The protection is typically manifested as both a reduction in abiotic stress symptoms and inhibition of pathogen growth (biotic stress), which can be phenotypically similar to pathogen-induced systemic acquired resistance (SAR) or induction of systemic resistance (ISR, Ross 1961). This PGPB effect has been demonstrated in different plant species, such as bean, carnation, cucumber, radish, tobacco, tomato, and in the model plant *A. thaliana* (Van Loon et al. 1998). Although it is well known that SAR or ISR triggered by PGPB confers resistance against pathogen-induced plant diseases, a few published reports suggest

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the role of PGPB as elicitors of abiotic stresses resistance in plants (Yang et al. 2009).

Plants synthesize a variety of chemical compounds, called phytohormones, which are actively involved in the regulation of plant growth and development (Arkhipova et al. 2007). IAA, a member of the phytohormones group, is generally considered to be the most important native auxin. These hormones can directly, or, in concert with other bacterial secondary metabolites, stimulate plant growth, usually in a concentration-dependent manner (Dimkpa et al. 2009a). Many reports have indicated that the main phytohormone classes (auxins, cytokinins, gibberellins, abscisic acid and ethylene) are produced by plant growth promoting bacteria (Forchetti et al. 2007). Phosphorus is one of the major nutrients, second only to nitrogen, that is required by plants, and most of the phosphorus in the soil is present in the form of insoluble phosphates and cannot be utilized by plants. Low levels of soluble phosphate in soil can limit plant growth. However, some plant-growth promoting bacteria solubilize phosphate from either organic or inorganic bound phosphates, thereby facilitating plant growth. The ability of bacteria to solubilize mineral phosphates has been studied by agricultural microbiologists as it can enhance the availability of phosphorus for plant growth (Vassilev et al. 2006). Many bacteria also have the ability to produce metal-chelating substances, such as iron-chelating siderophores. Siderophore-producing bacteria have been shown to influence plant uptake of various metals, including iron, zinc and copper. Because microbial iron-siderophore complexes serve as an iron source for monocot and dicot plants, iron deficiency symptoms, genuine or metal induced, common in plants grown under high heavy metal concentrations can also be prevented (Dimkpa et al. 2008). Bacteria can affect the bioavailability of heavy metals which are toxic to plants in low concentrations, and make them unavailable to the phytopathogens (Dimkpa et al. 2009b).

PGPB genera include *Azospirillum*, *Enterobacter*, *Klebsiella*. *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Paenibacillus*, and some are members of the *Enterobacteriaceae* (Hayat et al. 2010). The genus *Paenibacillus* is a group of Gram-positive, spore-forming, rod-shaped, and facultative aerobic bacteria. Ash et al. (1993) proposed that members of "group 3" within the *Bacillus* genus should be transferred to a new genus, and *P. polymyxa* was proposed as a type species that could be separated from the genus *Bacillus*. Multiple *Bacillus* and *Paenibacillus* spp. can promote crop health in a variety of ways. In general, there are two ways to influence the antagonistic/plant growth-promoting potential: (1) by managing the indigenous microbial potential, for example by giving organic or inorganic amendments and (2) by applying autochthonous microorganisms as plant growth-promoting or biocontrol agents (Weller 2007). The use of PGPB as bio-protectors and/or plant growth stimulators is probably one of the most significant plant management tactics, mainly due to the growing necessity of sustainable agriculture that has focused on environmentally friendly practices, such as reducing the use of chemical fertilizers (Figueiredo et al. 2008).

Several bacteria candidates were isolated from Yongin forest soil and plant growth promoting traits such as IAA production, phosphate solubilization, and siderophore production were determined. Based on the screening tests, *Micrococcus yunnanensis* PGPB7 and *Paenibacillus barengoltzii* PGPB8 was selected as PGPB candidates and tested on *A. thaliana*. However, based on the results, our focus in this report was *P. barengoltzii* PGPB8 which was previously reported as a novel species, *P. yonginensis* DCY84^T (Sukweenadhi et al. 2014). Until now, with the exception of *P. polymyxa*, very few reports have examined plant growth promoting activity in the *Paenibacillus* genera. This paper reports changes in *A. thaliana* gene expression after inoculation with plant growth promoting bacteria, against several abiotic stress conditions, such as salinity, drought and heavy metal stress. The results indicate that genes and/or gene classes related to plant defenses against abiotic and biotic stress may be co-regulated. Some tests were also conducted with *Oryza sativa* L. as a target plant to show the practical use of this PGPB candidate in the crop improvement on coastal area.

2. Materials and methods

2.1. Molecular characterization

The genomic DNA of isolated strains was extracted using a DNA isolation kit (Gene All Biotechnology, Republic of Korea). The 16S rRNA gene was amplified using the universal bacterial primer sets including 27F/1492R (Lane 1991) and 518F/800R (Weisburg et al. 1991). The purified PCR product was sequenced by Genotech (Daejeon, Republic of Korea). Seq-Man software version 4.1 (DNASTAR.) was used to compile the 16S rRNA sequence of isolated strains. Each strain's sequence was obtained and compared with those in public databases using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) and the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.2. In vitro assessment of plant growth promoting traits

The method described by Glickmann and Dessaux (1995) was used to check IAA production. Some modifications for *in vitro* IAA production were performed using modified King B broth (Casein 10 g/L, Peptone no.3 10 g/L, dipotassium phosphate 1.5 g/L, magnesium sulfate 1.5 g/L) media with and without additional L-tryptophan (3 g/L), as described by Shokri and Emtiazi (2010). After 6 days of incubation, the production of IAA was measured using the colorimetric method (Salkowski reagent). Qualitative testing of phosphate solubilizing ability of isolated strains was checked by plate screening methods using the media manually prepared as described by Pikovskaya (1948). *Pseudomonas* Agar F (Difco) medium (Glickmann and Dessaux 1995) supplemented with a chrome azurol S complex [CAS/iron(III)/hexadeciltrimethyl ammonium bromide] was used to assess siderophore production capacity, as described by Schwyn and Neilands (1987).

2.3. Bacteria inoculation with Arabidopsis thaliana

A. thaliana ecotype Columbia (Col-0) was used in this experiment. Seeds were surface-sterilized in 1.125% (w/v) NaOCl solution for 15 min and washed at least 5 times with sterilized distilled water. Seeds were directly sown on the sterilized soil. After germination, the plants were replanted separately and grown for 2 weeks in a growth chamber at 22 °C with a 16-h light photoperiod. *M. yunnanensis* PGPB7, *P. barengoltzii* PGPB8 which was designated as novel species *P. yonginensis* DCY84^T by Sukweenadhi et al. (2014), and *P. polymyxa* KACC 10485^T (as positive control) were selected to inoculate *A. thaliana* plants. Each bacteria strain was grown on TSB media at 30 °C to late log phase. Culture broth was centrifuged at 3000 × *g* for 15 min and the precipitated cells were dissolved in saline water. The bacteria suspension was centrifuged at 3000 × *g* for 15 min again and dissolved in saline water until reaching 10^8 CFU/mL. After 2 weeks of growth, *A. thaliana* were inoculated by soaking their roots in a 10^8 CFU/mL bacteria suspension for 24 h; mock plants and negative control plants were soaked in saline water.

2.4. Abiotic stresses

After 1 week of inoculation with bacteria, abiotic stress treatment (saline stress, drought stress, and heavy metal stress) was given separately to the negative control, positive control

(*P. polymyxa* KACC 10485^T), *M. yunnanensis* PGPB7 and *P. yonginensis* DCY84^T treated plants while the mock plant was not given any stresses. Saline stress was conducted by watering *A. thaliana* with a 200 mM NaCl solution. Drought stress was conducted by not watering *A. thaliana*. Heavy metal stress was conducted by watering *A. thaliana* with 1 M $Al_2(SO_4)_3$ solution. Duration of stress treatment was 5 days and 5 pot replications were conducted for each treatment group. The plant materials from all completed treatments were immediately frozen in liquid nitrogen and stored at −70 °C until the next analysis.

2.5. Quantitative real-time PCR

Total RNA was isolated from completely treated *A. thaliana* using the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The concentration of RNA was measured using a GE nano-value spectrophotometer. 2 μg of total RNA were reverse transcribed using the Power cDNA kit (Invitrogen) following the instruction given by the manufacturer, in a 20-μL total reaction volume to obtain the first strand of cDNA. Synthesized cDNA was diluted into 100 μL, and 3 μL of this dilution was used for quantitative real-time PCR (qRT-PCR). qRT-PCR was performed using a CFX Connect™ Real-time system (Bio-Rad Laboratories Inc., Hercules, CA, USA) with a 10-μL reaction volume using SYBR® Green Sensimix Plus Master Mix (Quantace, Watford, England) with the gene specific primers (Table 2). The housekeeping gene that encoded actin was used as a control. The thermal cycler conditions recommended by the manufacturer were used: 3 min at 95 °C, followed by 39 cycles at 95 °C for 10 s, annealing temperature of each primer pairs for 30 s, then ended with 95 °C for 10 s and 65–95 °C for 5 s with 0.5 °C increments for melt curve analysis. The fluorescent product was detected during the final step of each cycle. Amplification, detection, and data analysis were carried out on a CFX Manager™ Software version 3.1 (Bio-Rad Laboratories Inc., Hercules, CA, USA). To determine the relative fold-differences in template abundance for each sample, the Ct value for each gene expression were normalized to the Ct value for β-actin and calculated relative to a calibrator using the formula $2^{-\Delta\Delta Ct}$. Three independent experiments were performed.

2.6. Compatibility test with Xanthomonas oryzae pv. oryzae Philippine race 6 (PXO99)

To determine the potential use of PGPB candidates as biocontrol agents on rice plants (*O. sativa* L.), *M. yunnanensis* PGPB7 and *P. yonginensis* DCY84^T were cultured overnight until late log phase (10^8 CFU/mL). 10 μL of bacteria culture was dropped on TYE media (tryptone 10 g/L, Yeast extract 5 g/L, NaCl 5 g/L, pH 7.5) that contained 10^8 CFU/mL PXO99. *Escherichia coli* TOP10 strain was also used as a control. All strains were incubated at 30 °C for 16 h.

2.7. Virulence assay inoculation experiments using Oryza sativa L. var. japonica cv. Chuchung

Rice plants were grown in the greenhouse for 6 weeks and were transferred to the growth chamber for inoculation under the following conditions: 14-h days at 28 °C and 10-h nights at 24 °C, 90% relative humidity. *M. yunnanensis* PGPB7 and *P. yonginensis* DCY84^T strains were grown at 30 °C on TSB until late log phase. The culture broth was then centrifuged and resuspended in sterile saline water to a concentration of 10^8 CFU/mL. PXO99 were grown for 72 h at 28 °C on peptone sucrose agar with appropriate antibiotics and were resuspended in sterile distilled water to a concentration of 10^9 CFU/mL. Inoculation of *M. yunnanensis* PGPB7 and *P. yonginensis* DCY84^T strains was performed using the dipping method for 2 h, followed by a 1-day recovery period. Each strain was inoculated to *O. sativa* separately. After the recovery period, the PXO99 strains were inoculated using the scissor-clip method, cutting approximately 4 cm from the tip of fully expanded leaves with scissors dipped in the above bacterial suspensions (Kaufman et al. 1973). Lesion lengths were measured after 14 days. The results represent averages from three inoculated leaves per strain.

2.8. Bacteria inoculation to Oryza sativa L. var. japonica cv. Chuchung

M. yunnanensis PGPB7, *P. yonginensis* DCY84^T and *P. polymyxa* KACC 10485^T strains were grown at 30 °C on Trypticase soy broth until the late log phase. The culture broth was then centrifuged and resuspended in sterile saline water to a concentration of 10^8 CFU/mL. Chuchung rice cultivar seeds were dipped in the bacteria suspension for 24 h. Saline water was used as a control treatment. 50 rice seeds of each treatment were planted in a soil pot and incubated under the following conditions: 14-h days at 28 °C and 10-h nights at 24 °C, 90% relative humidity. The soil used in this experiment was collected from the coastal area of Suncheon, South Korea. Germination rate was recorded after 2 days of planting while the shoot length, shoot number, root length, root number, fresh weight, and dry weight were recorded after 2 weeks of growing.

3. Results

By distinguishing and grouping the colonies based on morphology differences, 10 PGPB candidate strains were selected from isolated strains; designated as PGPB1, PGPB2, PGPB3, PGPB4, PGPB5, PGPB6, PGPB7, PGPB8, PGPB9 and PGPB10. The 16S rRNA sequence of each isolate was compared with the EzTaxon database. Among the 10 bacteria isolates, 4 isolates belonged to the genus *Bacillus*, 3 isolates belonged to the genus *Paenibacillus*, 2 isolates belonged to the genus *Brevibacillus* and 1 isolate belonged to the genus *Micrococcus* (Table 1). Some of those isolates produced IAA in media without tryptophan and the others only showed increasing IAA production on modified media with additional L-tryptophan. However, some others did not show any significant IAA using both kind of media, which indicated that those isolates did not have the ability to produce IAA. As shown at Table 1, the highest IAA producer in media with and without supplemented L-tryptophan were *P. polymyxa* KACC 10485^T (KACC 10485^T) which produced 85.67 ± 5.70 μg/mL and 80.14 ± 0.69 μg/mL, followed by *P. yonginensis* DCY84^T (DCY84^T) which produced 72.83 ± 2.26 μg/mL and 69.96 ± 1.85 μg/mL then *M. yunnanensis* PGPB7 (PGPB7) which produced 52.94 ± 1.30 μg/mL and 49.48 ± 1.05 μg/mL, respectively. For the phosphate solubilization test, the clear halo region around the colonies grown in opaque medium indicated positive results. Only 4 out of 11 tested strains showed positive results which are KACC 10485^T, DCY84^T, PGPB7 and PGPB9 (Table 1). The positive siderophores production results were confirmed by the development of yellow/orange halos surrounding those colonies. All bacteria isolates were able to produce siderophores although some of them had weak production (Table 1).

Based on the screening results of plant growth promoting traits, DCY84^T and PGPB7 were used to evaluate putative plant-growth-promoting effects using *A. thaliana* and KACC 10485^T was used as positive control. In pilot experiments, we observed that *A. thaliana* treated with *Paenibacillus* strains showed mild stress symptoms: both its leaf and root were clearly stunted compared with mock-treated control plants (Fig. 1). After inoculation of PGPB candidates and 1 week recovery time, *A. thaliana* was challenged independently to several abiotic stress treatments including salinity (200 mM NaCl), drought and heavy metal (1 M $Al_2(SO_4)_3$). During 5 days of continuous stress, the morphological appearance

Table 1
In vitro plant growth promoting traits of PGPB isolates and *Paenibacillus polymyxa* KACC 10485^T.

Isolates	Pairwise similarity of the 16S rRNA sequence		IAA production (μg/mL)		Phosphate solubilization[a]	Siderophore production[a]
	Nearest strain	Similarity (%)	w/o L-tryptophan	With L-tryptophan		
PGPB1	*Bacillus sonorensis* NRRL B-23154	99.9	0.55 ± 0.17	1.17 ± 0.14	(−)	w
PGPB2	*Bacillus subtilis* subsp. *inaquosorum* BGSC3A28	99.9	2.66 ± 0.32	12.24 ± 0.46	(−)	w
PGPB3	*Bacillus methylotrophicus* CBNB 205	99.9	1.13 ± 0.40	12.20 ± 0.50	(−)	w
PGPB4	*Bacillus oleronius* DSM 9356	99.0	13.18 ± 2.48	22.63 ± 1.18	(−)	(+)
PGPB5	*Brevibacillus borstelensis* NRRL NRS-818	99.8	24.25 ± 0.87	26.12 ± 0.92	(−)	w
PGPB6	*Brevibacillus brevis* NBRC 15304	98.8	32.57 ± 1.85	39.09 ± 1.05	w	(+)
PGPB7	***Micrococcus yunnanensis* YIM 65004**	**99.4**	**49.48 ± 1.05**	**52.94 ± 1.30**	**(+)**	**(+)**
PGPB8 = DCY84^T	***Paenibacillus barengoltzii* KACC 15270^T**	**96.9**	**69.96 ± 1.85**	**72.83 ± 2.26**	**(+)**	**(+)**
PGPB9	*Paenibacillus cookii* LMG 18419	99.6	36.76 ± 0.45	38.66 ± 2.64	(+)	w
PGPB10	*Paenibacillus macerans* IAM 12467	99.7	31.47 ± 0.69	33.98 ± 2.71	(−)	(+)
***P. polymyxa* KACC 10485^T**			**80.14 ± 0.69**	**85.67 ± 5.70**	**(+)**	**(+)**

[a] (+) = positive result, (−) = negative results, w = weak results, because the positive result appeared after more than 1 week.
DCY84^T = on the basis of the phenotypic characteristics, genotypic analysis, and chemotaxonomic characteristics, strain PGPB8 is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus yonginensis* sp. nov. is proposed. The type strain is DCY84^T = KCTC 33428^T = JCM 19885^T (Sukweenadhi et al. 2014).

of the negative control *A. thaliana* plant showed severe damaged due to the given stress, compared to the mock *A. thaliana* plant. The stress symptoms varied but included smaller grown leaves, shrunk leaves or yellow-colored leaves (Fig. 1). Plants treated with PGPB7 also showed similar symptoms, although the NaCl stressed plant looked slight better than the negative control (Fig. 1). However, the *Paenibacillus*-treated plants showed better morphological appearance in all of the stress situations and had similar look to

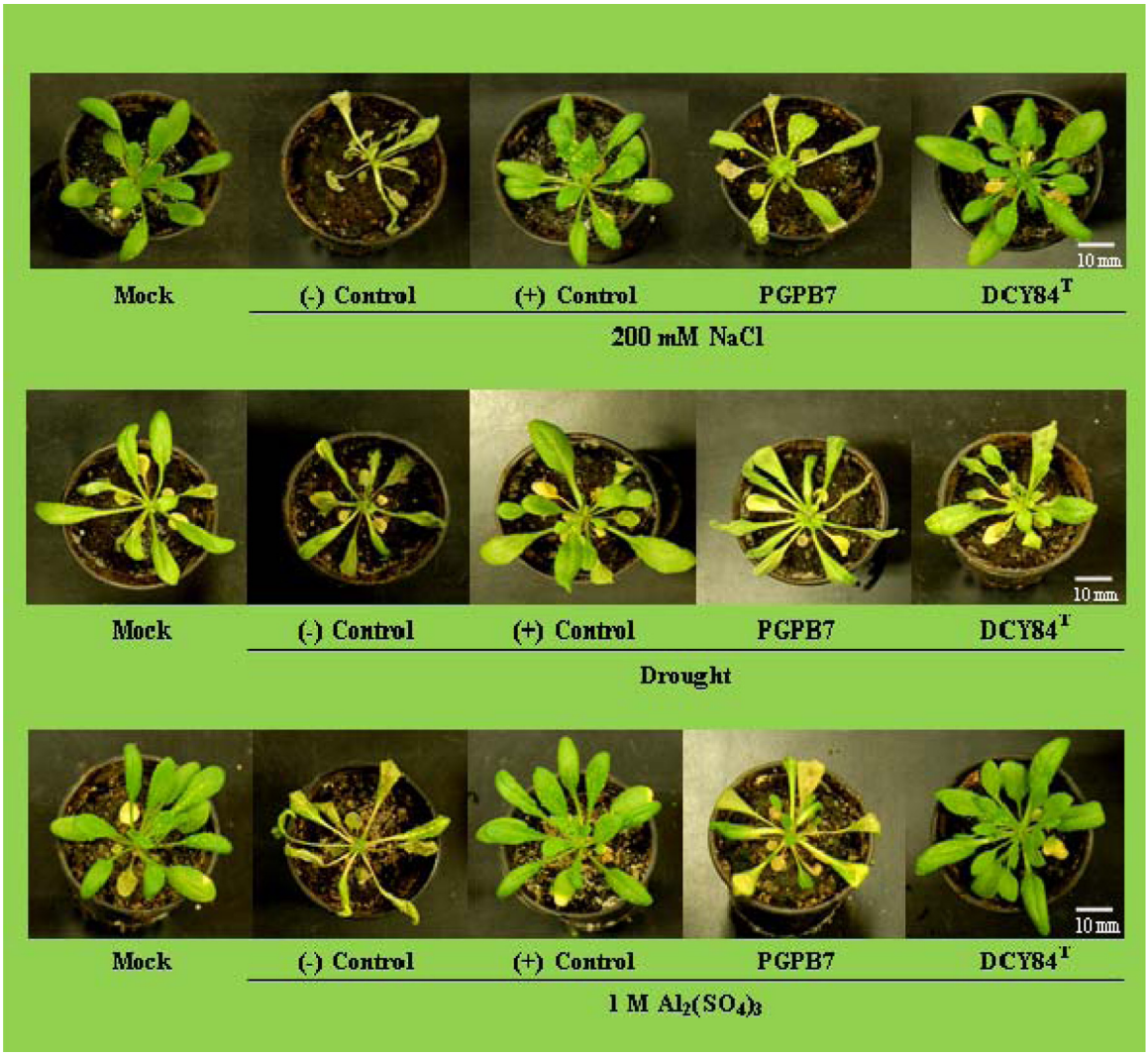

Fig. 1. Representative results of morphological comparison of *Arabidopsis thaliana* co-cultured with PGPB isolates under various abiotic stress after 5 days of treatment; Mock = no inoculant and no stress; (−) Control = no inoculant; (+) Control = *Paenibacillus polymyxa* KACC 10485^T, PGPB7 = *Micrococcus yunnanensis* PGPB7, DCY84^T = *Paenibacillus yonginensis* DCY84^T.

Table 2
Primers.

Primer	Sequence (5′–3′)	Temp. (°C)	Annealing Temp. (°C)
Real time PCR primer to determine transcript levels under salinity stress			
AtRSA1-F	GAAGTTCAGTGAGTGGTGGCGCAGAAGGAG	65.1	58
AtRSA1-R	GGTGAATCAGGTAAAGTAGGGACATATC	56.5	
AtVQ9-F	AATCACCGGTCTCTTCGTACAT	55.7	58
AtVQ9-R	GAGGTGAGACCAAAGGAGCTAA	56.2	
AtWRKY8-F	ATGATCTCTTCCGTGTGCCA	56.3	58
AtWRKY8-R	ATCATCAAGGCTCTTGTTTGAAGA	54.7	
Real time PCR primer to determine transcript levels under drought stress			
AtERD15-F	CCAGCGAAATGGGGAAACCA	58.2	60
AtERD15-R	ACAAAGGTACAGTGGTGGC	55.2	
AtRAB18-F	CATGCCATGGCGTCTTACCAGAACCGTCC	65.8	60
AtRAB18-R	TTTACTGCAGTTAACAACGGCCACCACCGGGAAGC	68.2	
AtLT178-F	CTCAGAAACTTTCAAAGAGCTTAGAAAA	54.1	60
AtLT178-R	AAGAGAGCGTTGGTTTGTACTTTGT	57.0	
Real time PCR primer to determine transcript levels under aluminum stress			
AtAIP-F	TCGTCGAGAAAAATCCATCC	52.4	55
AtAIP-R	CAATGGAACCTTGCCAACTT	53.8	
AtALS3-F	TGTTTCCCGATCGTTTCTTC	52.8	55
AtALS3-R	TAATCCGGCCACGTACTTTC	54.7	
AtALMT1-F	GAGAGCTCGGTGAAAAGGTG	55.7	55
AtALMT1-R	CGTGGTTTTCTGGTGGATCT	55.0	
Housekeeping gene			
AtActin2-F	GTGTGTCTTGTCTTATCTGGTTCG	55.5	55, 58, 60
AtActin2-R	AATAGCTGCATTGTCACCCGATAC	57.2	

the mock plant (Fig. 1). The observations showed that inoculation of *A. thaliana* by DCY84^T provides significant resistance to salinity, drought, and heavy metal treatment. All plant RNA were isolated, synthezised to cDNA and used as a template for qRT-PCR to verify the relative transcription level under different kinds of stress. qRT-PCR was conducted using several specific primers designated for each kind of stress and *AtActin2* gene served as an internal control (Table 2).

AtRSA1, *AtVQ9* and *AtWRKY8* were used to determine the response to saline. *A. thaliana* treated with DCY84^T and KACC 10485^T showed higher *AtRSA1* transcription levels (around 1.5-fold) compared to the negative control plant which was not treated with any PGPB. Those strains also showed significant increase of *AtWRKY8* (around 1.5-fold) and decrease of *AtVQ9* (around ½-fold). However, *A. thaliana* treated with PGPB7 showed slightly higher *AtRSA1* transcription levels and no different results in both the *AtWRKY8* and *AtVQ9* transcription level compared to the negative control (Fig. 2). *AtERD15*, *AtRAB18* and *AtLT178* were selected to determine *A. thaliana* responses against drought stress. All of those genes were upregulated in stressed plant (8–10-fold) compared to the non-stressed plant. Moreover, *A. thaliana* treated with DCY84^T and KACC 10485^T showed higher transcription levels (1.4–2.2-fold) compared to negative controls (Fig. 2). Aluminum stress response in *A. thaliana* was assessed using *AtAIP*, *AtALS3*, and *AtALMT1* because all of those genes are enhanced by Al treatment. In this aluminum stress treatment, DCY84^T and KACC 10485^T showed higher transcription levels (around 1.5-fold) compared to negative controls in all aluminum responsive genes that were assessed. In the other hand, *A. thaliana* treated with PGPB7 showed no significant difference in the amount of *AtAIP*, *AtALS3* and *AtALMT1* transcription levels compared to the negative control.

Compatibility test with PXO99 showed positive results which were indicated by clear zone formation around colonies of PGPB7 and DCY84^T but not on the plates for *E. coli* TOP10. Both results were not very strong but the PGPB7 formed very distinguishable clear zone (Supplementary Fig. S1A). Furthermore, *O. sativa* treated with PGPB7 or DCY84^T showed light PXO99 infection symptoms compared to the control, which was measured by averaging the lesion length of rice leaves. The average lesion length for the control was 13.2 ± 0.64 cm. However, the average lesion length for PGPB7 and DCY84^T were 8.62 ± 0.35 cm and 3.34 ± 0.21 cm, respectively (Supplementary Fig. S1B). This result showed distinct symptoms of PXO99 infection between those that were given PGPB treatment and the control as provided in the representative result, as DCY84^T showed the healthier symptoms of *O. sativa* infected by PXO99 (Supplementary Fig. S1C).

Supplementary Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.micres.2015.01.007.

DCY84^T was also tested to determine rice seedling growth ability in coastal soil collected from Suncheon coastal. Rice seeds treated with *Paenibacillus* were found to germinate slower than other treated seeds, although the seeds had a higher germination rate. After 2 weeks, DCY84^T was found to be able improving rice plant growth (Fig. 3). Several growth parameters showed that DCY84^T had the highest plant growth promoting activity, which was slightly higher than KACC 10485^T, when both of them compared to mock plants (Table 3). Chemical analysis of Suncheon soil is provided in the Supplementary Table S1.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.micres.2015.01.007.

4. Discussion

PGPB have various plant growth promoting traits which was recently discussed by Ahemad and Kibret (2014). Among those traits, IAA production, phosphate solubilization and siderophore production were commonly found with PGPB. The IAA production by bacteria can vary among different species or strains, and is also influenced by growth stage, substrate availability and culture condition. Therefore, specific availability of substrates as phytohormones precursors, such as L-tryptophan for IAA, is a major factor determining the degree of bacterial stimulation of plant growth (Rajkumar et al. 2005). Based on the results, some isolated strains were able to produce IAA even without additional L-tryptophan, indicating its ability as strong IAA producer. Meanwhile, some strains were only able to produce IAA if the media supplemented with L-tryptophan, indicating its weak ability as IAA producer. Higher concentrations of phosphate solubilizing bacteria are commonly found in the rhizosphere in comparison with

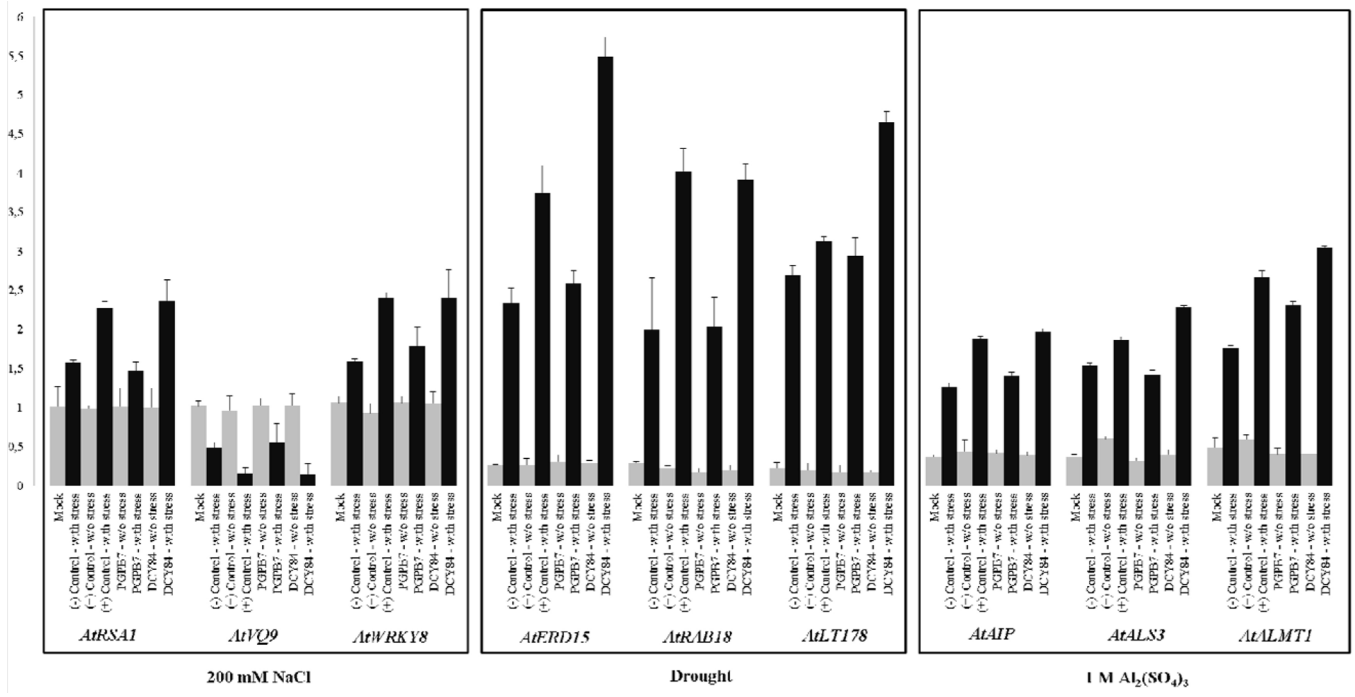

Fig. 2. Relative quantities of *Arabidopsis thaliana* co-cultured with PGPB isolates under various abiotic stress after 5 days of treatment; Mock = no inoculant and no stress; (−) Control = without inoculant; (+) Control = *Paenibacillus polymyxa* KACC 10485^T, PGPB7 = *Micrococcus yunnanensis* PGPB7, DCY84^T = *Paenibacillus yonginensis* DCY84^T.

Table 3
Effects of PGPB inoculations on *Oryza sativa* L. Chuchung cultivar growth promotion after 2 weeks.

Growth parameter	Treatment			
	(−) Control	(+) Control	PGPB7	DCY84^T
Germination rate (%)	22	60	32	68
Longest shoot (mm)	125.8 ± 14.19	172.0 ± 16.87	144.7 ± 9.64	183.4 ± 7.66
Number of shoots	2.0 ± 0.10	2.1 ± 0.20	2.1 ± 0.07	2.1 ± 0.10
Longest root (mm)	39.3 ± 3.88	64.9 ± 4.07	43.1 ± 4.23	71.3 ± 2.20
Number of roots	6.6 ± 0.20	7.4 ± 0.29	7.1 ± 0.25	7.8 ± 0.15
Fresh weight (mg)	79.74 ± 4.37	96.97 ± 3.59	84.56 ± 2.98	101.24 ± 2.59
Dry weight (mg)	31.90 ± 3.76	38.79 ± 2.74	35.82 ± 2.11	40.49 ± 2.70

(−) Control = no inoculant, (+) Control = *Paenibacillus polymyxa* KACC 10485^T, PGPB7 = *Micrococcus yunnanensis* PGPB7, DCY84^T = *Paenibacillus yonginensis* DCY84^T.

non-rhizospheric soil or free-living bacteria (Hayat et al. 2010). In the rhizosphere, species of the genus *Bacillus* and *Paenibacillus* are involved in atmospheric nitrogen fixation, solubilization of soil phosphorus and uptake of micronutrients, and production of phytohormones and antimicrobial metabolites as reported by Govindasamy et al. (2011). On the siderophore production test, 5 isolated strains showed positive results while others showed weak results. The ability of some bacteria to protect against nickel, lead, or zinc toxicity has previously been shown to be related to the production of siderophores (Siddiqui 2005). It has often been assumed that competition for Fe in the rhizosphere is controlled by the Fe affinity of the siderophores, whereby the ligands produced by the biocontrol agent have higher formation constants than those of the pathogen (Loper and Henkels, 1995). All of these results reinforced a previous report about the *Micrococcus* strain NII-0909 (isolated from a Western Ghats forest) which possessed multiple plant growth traits, such as P-solubilization, IAA, and siderophore production (Dastager et al. 2010). Furthermore, it was also consistent with previous studies of *P. polymyxa* (Phi et al. 2010), *P. rhizosphaerae* TGX5E, and *P. favisporus* TG1R2 (Bidondo et al. 2011).

A. thaliana was chosen as the model plant because it provides a good experimental system for genetic studies. Furthermore, it has a great number of sequence entries in databases, easily facilitating identification of genes whose expression might be altered by the PGPB. This phenomenon was confirmed the previous report on *P. polymyxa* (Timmusk and Wagner 1999). Previously reported nuclear-localized calcium-binding protein, AtRSA1, was found to regulate the transcription of several genes involved in the detoxification of reactive oxygen species generated by salt stress. It was also found that *AtRSA1* (At3g06590) mutant plants were hypersensitive to NaCl but not to LiCl, CsCl, or general osmotic stress (Guan et al., 2013). *AtWRKY8* (At5g46350) was mainly responds to high salinity compared to others abiotic stress treatments. It was also determined that *AtWRKY8* only specifically interacted with *AtVQ9* (At1g78310) and the interaction decreased the DNA-binding activity of *AtWRKY8*. *AtVQ9* protein was exclusively localized in the nucleus, had higher expression at roots and mutation enhanced salt stress tolerance, indicating that *AtVQ9* acts antagonistically with *AtWRKY8* to mediate salt stress responses (Hu et al. 2013). As previously described by Guan et al. (2013), *AtRSA1* senses salt-induced changes in nuclear free calcium and interacts with a bHLH transcription factor, *AtRITF1*, which may be phosphorylated by nuclear-localized mitogen-activated protein kinases (MAPKs). The *AtRSA1-AtRITF1* complex controls gene expressions which are responsible for Na^+ homeostasis under salt stress and detoxification of salt-induced reactive oxygen species (Guan et al., 2013). Both *AtWRKY8* and *AtVQ9* maintain ion homeostasis, especially at a lower cytosolic Na^+/K^+ ratio which is a critical determinant of

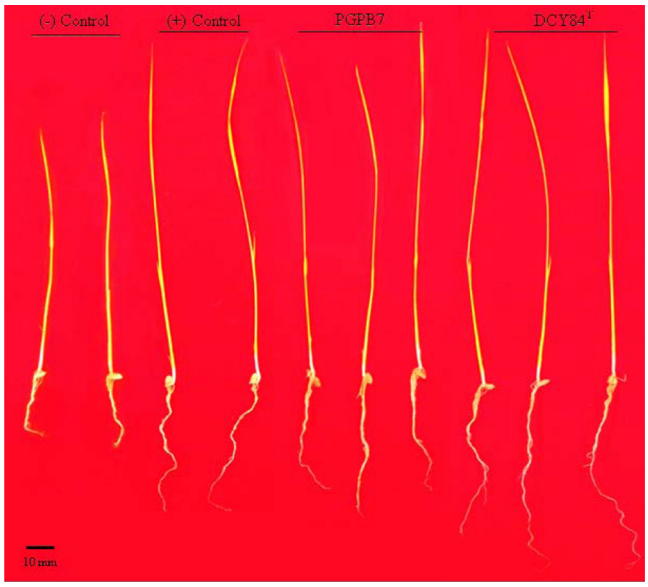

Fig. 3. Representative results of 2-week-old *Oryza sativa* L. Chuchung cultivar seedlings after treatment with PGPB and grown on salty soil; (−) Control = no inoculant; (+) Control = *Paenibacillus polymyxa* KACC 10485^T, PGPB7 = *Micrococcus yunnanensis* PGPB7, DCY84^T = *Paenibacillus yonginensis* DCY84^T.

salt adaptation in plants (Hu et al. 2013). As those *AtRSA1*, *AtVQ9* and *AtWRKY8* selected as the saline responsive gene, the increasing transcription level of both *AtRSA1* and *AtWRKY8*, also decreasing transcription level of *AtVQ9* (*AtWRKY8* antagonist) indicating how the plant become more responsive against salt stress condition, as observed at DCY84^T and KACC 10485^T treated *A. thaliana* (Fig. 2). However, trancription level of *AtRSA1* confirmed that PGPB7 can only confer mild salt tolerance to *A. thaliana*.

AtERD15 (At2g41430) is rapidly induced in response to various abiotic and biotic stress stimuli in *A. thaliana*. Overexpression of *AtERD15* reduced the abscisic acid (ABA) sensitivity, resulting in decreased drought tolerance in response to this hormone. In comparison, RNAi silencing of *AtERD15* resulted in ABA-hypersensitive plants that showed improved tolerance to drought (Kariola et al. 2006). Several mutations in genes encoding proteins involved in RNA metabolism, such as the mRNA cap-binding ABH1, have been shown to affect ABA sensitivity in *A. thaliana* (Kuhn and Schroeder, 2003). During vegetative growth, ABA is a central regulator of plant adaptation to environmental stresses, such as drought and high salinity. ABA causes stomata1 closure as the results of drought stress response by inducing changes in the turgor of the two surrounding guard cells. These changes are an example of the rapid effects of ABA, due to changes in gating properties of ion channels (Zhu, 2002). However, higher transcription expression result of *AtERD15* contradicted the morphological results, because high levels of *AtERD15* will reduce ABA sensitivity. Thus, the drought tolerance should be reduced and plants should experience severe damage due to the stress conditions as shown by the negative control but not shown by PGPB treated *A. thaliana* (Fig. 1). It raised some possibility that *A. thaliana* tried to compensate for the reduced ABA sensitivity by producing more ABA. RAB18 mRNA was reported accumutale in plants exposed to low temperature, drought, or exogenous ABA (Welin et al. 1994), but the primary function of *AtRAB18* is in tolerance to cellular dehydration rather than being directly involved with freezing tolerance (Lång and Palva 1992). In contrast, *AtLTI78* is strongly induced by low temperature, but weakly induced by ABA and drought (Nordin et al. 1993). Increasing transcription levels of *AtRAB18* confirmed the cellular dehydration due to the drought stress, while upregulated *AtLTI78* confirmed the previous hypothesis that *A. thaliana* produced more ABA during drought stress. Because the *AtERD15* ABA signaling pathway by abiotic stress might be correlated with the biotic stress pathway as proposed by Kariola et al. (2006), the higher relative *AtERD15* transcription level in the PGPB-treated plant might be attributable to the PGPB inoculation. These results may suggest that PGPB treatment can trigger both biotic and abiotic stress-related defense pathways, or that pathways are actually overlapping. The *AtRAB18* (At1G43890) and *AtLT178* (At5g52310) genes show elevated expression levels in response to drought stress and/or ABA stimulus (Fig. 2). In this case, DCY84^T showed an ability to confer drought resistance on *A. thaliana* by inducing *AtRAB18* and *AtLT178*.

AtAIP (At5g19140) and *AtALS3* (At2g37330) were found to be the most highly expressed in the leaf whereas *AtALMT1* (At1g08430) was found to be abundant in the root, based on the *A. thaliana* EFP Browser (http://bar.utoronto.ca/eip/cgi-bin/efpWeb.cgi; Winter et al. 2007) and *AtAIP* was confirmed to be in the plasma membrane (Hall 2002). *AtALS3* may have function to redistribute accumulated Al away from sensitive tissues to less sensitive tissues. One study showed that *AtALS3* knockout plants suffered from the accumulation of Al in inappropriate tissues, which subsequently resulted in severe inhibition of root and shoot growth (Larsen et al. 2005). *AtALMT1* can facilitate Al-activated root malate exudation (Hoekenga et al. 2006), *via* STOP1, a putative zinc finger transcription factor involved in low-pH resistance and Al tolerance in *A. thaliana* (Iuchi et al. 2007). All of these *AtAIP*, *AtALS3* and *AtALMT1* were increased indicateing that DCY84^T can induce aluminum tolerance in *A. thaliana*.

Plant diseases are among the main constraints that affect the production and productivity of crops in terms of quality and quantity. *Xanthomonas oryzae* pv. *oryzae* is a pathogenic bacterium which causes serious blight of rice, other grasses, and sedges. Because rice is a major crop plant in Asia, chemicals used to mitigate the menace of crop diseases have become an increasing issue and the use of biocontrol agents to suppress the disease causing activity of plant pathogens is becoming increasingly important (Agrios 2005). Ecological niche and substrate competition are widely recognized mechanisms of biocontrol mediated by PGPB. These mechanisms allow for inhibitory allelochemical production and ISR in host plants to a broad spectrum of pathogens (Weller 2007) and/or abiotic stresses (Mayak et al. 2004). Timmusk and Wagner (1999) inoculated *A. thaliana* with *P. polymyxa* then challenged it with *Erwinia carotovora* (biotic stress) or drought (abiotic stress). Quantification of mRNA levels showed increases in both the drought responsive gene and the biotic stress pathway; this showed that *P. polymyxa* caused mild biotic stress. Those results indicated that genes and/or gene classes related to plant defenses against abiotic and biotic stress may be co-regulated (Timmusk and Wagner 1999). DCY84^T, which induced relative transcription levels of several abiotic stress responsive genes, also showed plant resistance to biotic stress conditions of *Xanthomonas oryzae* pv. *oryzae* (PXO99) infection based on both compatibility test and virrulence assay (Supplementary Fig. S1).

Salt stress is an important environmental factor that significantly limits crop plant productivity worldwide. Salt concentration in a soil is measured in terms of its electrical conductivity (EC); the SI unit of electrical conductivity is dS m^{-1}. The most widely accepted definition of a saline soil has been adopted from FAO as one that has an EC of 4 dS m^{-1} or more (Yadav et al. 2011). Traditionally, 4 levels of soil salinity based on saline irrigation water have been distinguished; low salinity (EC less than 0.25 dS m^{-1}); medium salinity (0.25–0.75 dS m^{-1}); high salinity (0.75–2.25 dS m^{-1}), and very high salinity with an electrical conductivity exceeding 2.25 dS m^{-1} (US Salinity Laboratory Staff, 1954). However, since salt stress depends on species, variety, growth stage, environmental factors, and nature of the salts, it is

difficult to define saline soils precisely. According to the classification of crop tolerance to salinity, the rice crop is within the sensitive division from 0 dS m^{-1} to 8 dS m^{-1} (Maas, 1986). For further application, DCY84^T also tested as plant strengthener, using rice and sample soil from Suncheon coastal area. Better growth parameter indicating that DCY84^T can help the rice seed to germinate and grow on Suncheon coastal soil which considered as medium saline. These preliminary test results can be used as considerations before conducting trial on a rice field.

5. Conclusion

Two PGPB candidates were selected from the 10 strains isolated from Yongin forest soil to determine several *in vitro* plant growth promoting traits such as IAA production, phosphate solubilization, and siderophore production. Further inoculation of *A. thaliana* showed that DCY84^T can promote plant tolerance against salt, drought, and aluminum stress, which was supported by several stress responsive genes. *In vitro* compatibility testing and a virulence assay against PXO99 also showed promising results for DCY84^T. Moreover, a trial that used a Chuchung rice cultivar grown on coastal soil from Suncheon province (34°51′54.24″ N, 127°29′18.39″ E) also led to better germination rates and growth parameters. Based on the results of this study, DCY84^T is a promising strain that can be used to develop microbial inoculants for crop improvement. Additional analyses are necessary, however, to determine the plant growth promoting mechanism of this species.

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