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Original Article

Biohardening of *Arabidopsis thaliana* Seeds and Seedlings with Fraser Photinia Associated Bacterium (PGB_invit) in *In vitro* Conditions

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Abstract

The aim of this study was to analyze possible positive effects of putatively endophytic PGPB (PGB_invit), which was isolated from long-term *in vitro* cultured fraser photinia microshoots, on seed and 7-day old seedling stages of *Arabidopsis thaliana*. Seeds and *in vitro*-germinated seedlings were inoculated with 10^7 CFU/mL and 10^8 CFU/mL active (A) and inactive (I) endophytic bacterial populations along with their mix compositions (A+I) and suspended in MPYE broth together with their controls (untreated ones). 14 days old seedlings were evaluated for various plant growth parameters [i.e., shoot and root fresh weight (FW), shoot length (SL), shoot and root dry weight (DW), root length (RL) and photosynthetic pigments including chlorophyll *a, b* and carotenoids of plantlets] as well as endophytic and rhizospheric trait of bacteria. Positive effects of inactive and active bacterium on FW, DW and photosynthetic pigments for 7-day inoculated seedlings were recorded whereas an increase in photosynthetic contents for seed stage inoculations was observed. Rhizospheric and endophytic colonization of the bacteria was confirmed by PCR with the presence of *virD1* gene, which is previously recorded to be existed in the plasmid of bacterium after inoculation (Şeker *et al*., 2017). Overall, these results demonstrated that this peculiar putative endophytic bacterium being beneficial in active and even more useful in inactive form for *A. thaliana* when optimum conditions and concentrations are used. Moreover, presence of *virD1* gene suggested its potential possibility to be used in bioengineering along with various other beneficial PGPR features as biofertilizer.

Keywords: *Arabidopsis thaliana*; endophytic bacteria; plant-bacteria interactions; *virD1* gene

Introduction

Plant growth promoting bacteria (PGPB) are among the beneficial interactions between plant and microorganisms which colonize the rhizosphere or internal tissues (endophytes) of many plant species and induce positive effects on physiology of plants such as not only increased plant growth but also improved tolerance to biotic (caused by fungi, bacteria, viruses and nematodes) and abiotic stresses (Poupin *et al*., 2013). Especially the endophytes took a lot of attention in recent years as they are still being a relatively untapped source of novel natural products. Thus, many plant species are being monitored for the diversity of endophytic bacteria within their tissues and consequently, new endophytes are continuously being reported (Hallmann and Berg, 2006; Rosenblueth and Martinez-Romero, 2006; Ryan *et al*., 2008).

It is well-known that beneficial endophytic bacteria could promote plant growth and yield either directly by i.e., i) nitrogen fixation, ii) solubilization of phosphorus, iii) sequestering of iron by production of siderophores, iv) production of phytohormones such as auxins, cytokinins, gibberellins and v) lowering of ethylene concentration (Kloepper *et al*., 1989; Glick, 1995; Glick *et al*., 1999) or indirectly by i.e., i) antibiotic production, ii) synthesis of antifungal metabolites, iii) depletion of iron from the rhizosphere, iv) competition for colonization on roots, v) production of fungal cell wall lysing enzymes, and vi) induced systemic resistance (Weller and Cook, 1986; Dunne *et al*., 1993; Kloepper *et al*., 1988; Liu *et al*., 1995; Glick *et al*., 1999).

There are several variables that effect the plant endophytes such as the growth stage of plant, type of plant tissue analyzed, plant health, nutritional condition of soil including pH and moisture content, temperature, altitude etc. and sometimes even the type of plant under consideration (in case of testing one endophyte on another

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plant) (Hardoim *et al*., 2008). Therefore, in order to have more understanding about various types of endophytes found in a single plant and their associations with plant, it is important to carry out broad screening techniques initially in model plants such as *A. thaliana*. For instance, Poupin *et al*. (2013) studied the effects of the PGPR bacterial model *Burkholderia phytofirmans* PsJN on the whole life cycle of *A. thaliana*. Their studies revealed that at different physiological stages of plant, strain PsJN colonized the roots of *A. thaliana* both rhizospherically and endophytically while early ontogeny also showed increased several growth parameters and accelerated growth rate of the plants. Inoculations of *A. thaliana* with *Paenibacillus yonginensis* DCY84 (T) and *Micrococcus yunnanensis* PGPB7 indicated that *P. yonginensis* DCY84 (T)-inoculated plants were more resistant to salinity, drought and heavy metal stresses than control plants (Sukweenadhi *et al*., 2015). A recent study also documented that PGPB strains *Pseudomonas simiae*, *Chryseobacterium polytrichastri* and *Burkholderia ginsengiterrae* co-inoculated with aluminum stressed *A. thaliana* were able to support the overall growth of plant by exhibiting plant growth promoting activities e.g. auxins and siderophores production and phosphate solubilization (Farh *et al*., 2017). Besides, some endophytic microbial contaminations in the plant tissue culture systems could also have beneficial influence on plant growth with supplying different additives and could have potential to use as bio-inoculants. Thus, isolation and characterization of these potential in *vitro* contaminants for production of biological compounds from plant cultures is becoming attractive for scientists. With this approach, our research group (Şeker *et al*., 2017) recently isolated a putatively endophytic bacterium (PGB_invit) that was detected in long-term *in vitro* cultured microshoots of fraser photonia. Phenotypical and biochemical properties of this bacterium, together with plant growth-promoting characteristics (PGPC) abilities including phytostimulation, biofertilization and hydrolytic activities, were determined and results showed that PGB_invit exhibited nitrogen-fixing ability as well as indole-3-acetic acid (IAA) and gibberellic acid (GA_3) producing capability. Gram-negative putative endophytic bacterium showed similarity to uncultured bacteria and Rhizobiales according to 16S rRNA sequencing and phylogenetic tree that was constructed by Neighbour Joining method. It is concluded that the bacterial strain with different PGPC can be considered as a beneficial microbe for the facilitation of fraser photinia growth. Thus, the aim of this study was to evaluate the possible interaction of PGB_invit with the model plant to determine whether this bacterium was i) host specific, ii) endophytic, iii) and had any beneficial effect on the two physiological stages of *A. thaliana in vitro*.

Materials and Methods

Plant material: Sterilization and in vitro seed germination

Arabidopsis thaliana Columbia (Col-0) seeds were used for all of the experiments. Seeds were surface sterilized with 70% v/v EtOH for 1-2 minutes followed by rinsing with 15% v/v bleach (NaOCl) for 10 minutes and washing 3 times for 5 minutes each with sterile distilled water. Afterwards, seeds were transferred to Petri plates (10 seeds/Petri plate) containing ½ MS (Murashige and Skoog, 1962) semi-solid medium. For seed germination, all Petri plates containing seeds were pre-placed at 4 °C in dark for ~3 days to synchronize germination before transferring to plant growth chamber where Petri plates were kept vertically at 22 °C with a photoperiod of 16 h of light (3000 lux) and 8 h of dark.

Cultivation of putative bacterium

The putatively endophytic bacterial strain, previously isolated and characterized from long-term *in vitro* cultured microshoots of fraser photinia (*Photinia* × *fraseri* Dress), was stored in glycerol at -80 °C (Şeker *et al*., 2017). PGB_invit was taken from -80 °C and pre-cultured in MPYE broth medium for 5-7 days followed by culturing in 250 mL of flasks. After 7-10 days, serial dilutions (up to 10^{-6}) of main culture were conducted in order to determine optimal concentration of bacterial inoculum.

Inoculation of PGB_invit to seed and seedlings

Two physiological stages of *A. thaliana* i.e. seeds stage and 7-day *in vitro* germinated seedlings stage, were inoculated with two different concentrations of active (A) PGB_invit, i.e. 10^7 CFU/mL (10^7) and 10^8 CFU/mL (10^8) using flood inoculation technique (Ishiga *et al.,* 2011). To assess the effect of inactivated bacterium, inoculums of same concentrations were heat inactivated separately at 95 °C for 20 minutes and named inactive (I) bacterium throughout the experiments. Moreover, A and I forms of bacterium were also tested together (A+I) at 10^7 and 10^8 concentrations for the possible effects on the two stages of *A. thaliana*. There were three replicate plates separately for each inoculum of bacterium along with a separate control. Plates were placed in plant growth chamber vertically at 22 °C with a photoperiod of 16 h of light and 8 h of dark.

Determination of influence of PGB_invit on A. thaliana

Fourteen (14) days old seedlings of *A. thaliana*, inoculated with different concentrations and populations of bacteria, were assessed for various growth parameters such as fresh (shoot and root) weight (FW), dry (shoot and root) weight (DW), root length (RL), shoot length (SL), photosynthetic pigments i.e. chlorophyll *a* (Chl a), *b* (Chl b) and carotenoids (Car). FW, SL and RL of individual seedlings were measured on the same day, whereas for DW, seedlings were to be incubated at 65 °C for at least 24 hours. For chlorophyll analysis, shoots were kept in acetone in 2 ml Eppendorf tubes at 4 °C for at least 24 hours and later, photosynthetic pigment analysis was carried out according to Lichtenthaler and Buschmann (2001) for wavelengths of 661.6 nm, 644.8 nm and 470 nm for Chl a, Chl b and Car, respectively. Similar procedure was followed for both the physiological stages where seeds were inoculated for 14 days for germination stage while 7 day-*in vitro* germinated seedlings were co-cultured for 7 days for seedling stage.

Assessment of bacterial existence inside seedlings

Various protocols were followed to evaluate endophytic and rhizospheric interaction of bacterium with plantlets. For endophytic interaction, surface sterilization of seedlings was carried out where randomly selected and named, endophytic active (E1), endophytic inactive (E2) and control (E3) seedlings from replicate plates, were separately weighed and suspended in 5% of hydrogen peroxide (H_2O_2) for 3 minutes in order to decontaminate roots from any associated bacterium. Later, seedlings were rinsed with sterile distilled water 3 times for 5 minutes each and crushed in sterile mortar pestle each separately with 1 ml of MPYE broth. This followed serial dilutions of crushed suspension from 10^0-10^{-4} in MPYE broth and 100 µl of each suspension was spreaded on MPYE agar plates with two replicates each. A validity check for sterilization protocol was also performed by inoculating last sterilization water from surface sterilization procedure.

Seedlings were randomly selected from replicate plates and weighed separately for rhizospheric active bacteria (R1), rhizospheric inactive bacteria (R2) and control (R3). Later, weighed seedlings were suspended, each separately, in bacterial growth medium i.e. MPYE and vortexed for 2 minutes in order to shed any possible bacterium associated with rhizosphere. R1, R2 and R3 were vortexed in place of sterilized crushed plant and broth mixture and same procedure was followed as for endophytic evaluation. All plates were incubated at 30 °C until the colonies appeared (normally in 7 days) and examined under light microscope for preliminary identification of putative endophytic bacterium by Gram-staining.

Plasmid DNA extraction

To confirm that the bacterial colonies found on MPYE agar plates belonged to PGB_invit, plasmid DNA from both the endophytic and rhizospheric cultures were isolated using Macherey-Nagel Nucleospin® Plasmid Extraction Kit. Concentration (ng/µl) of extracted plasmid DNA was measured on nanodrop and assayed by agarose gel analysis.

Verification of PGB_invit presence in A. thaliana by PCR

The presence of *A. rhizogenes virD1* gene in isolated PGB_invit was reported by Şah (2017). Thus, PCR amplification was conducted on plasmid DNAs isolated from both the endophytic and rhizospheric PGB_invit cultures. Each amplification mixture contained 1X FIREpol® PCR Mastermix (12.5 mM of $MgCl_2$), 0.2 µM of each primer and ~100 ng/µl of template DNA in a final volume of 20 µl. Following PCR conditions were optimized for the amplification of plasmid DNA: initial denaturation at 94 °C for 5 min., followed by 25 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 55 °C, and extension at 72 °C for 1 min with an additional extension at 72 °C for 7 min followed by a cool down and incubation at 4 °C. Universal forward (5-ATGTCGCAAGGCAGTAGGCCCACCT-3') and reverse primers (3'-CTACAAGGCGTCTTTCAGCAGCGAGC-5') were used for *virD1* amplification. All samples were run on a 1% agarose gel on gel electrophoresis system for 30 minutes at 80 volts where 1000bp plus DNA (24075 Sizer™, 144 ng/µl) was used as marker. Visualization was done using Gel Logic 200 Imaging system under UV light [Biotium Inc, Hayward CA, USA].

Data collection and statistical analysis

Each trial was done with at least 10 seeds/seedlings and the trials were repeated at least 3 times. Statistical analysis of *A. thaliana* growth parameters, subjected to different concentrations of putative endophytic bacterium, was carried out using LSD test. Significant error difference was $P \leq 0.05$.

Results

Effect of PGB_invit on in vitro seed germination

Experiments showed 100% germination rate for PGB-invit inoculated seeds, which indicated that there was no detrimental effect of bacterium on seed germination. For seed stage bacterial inoculations, both A 10^7 and 10^8 concentrations resulted in a decrease in FW of seedlings while I inoculation at 10^7 concentration showed statistically insignificant result in comparison to control (Table 1). However, when A and I form of bacterium were inoculated together, at both tested concentrations, the detrimental effect of the A form was not detected as the highest FW was obtained (47.08 mg) with A+I for 10^7. Similar effect was observed for DW too (Table 2). Moreover, no negative influence of bacterium in A form at 10^7 could be seen in SL (Table 3) but a decrease was observed with an increase in bacterial concentration. For A+I form of bacterium, a gradual positive influence was detected in which 10^8 resulted in the longest shoots (0.61cm). In case of RL, no significant effect of bacterium was evident for any replicates (Table 4).

In case of photosynthetic pigments, a significant positive effect was observed for A form of both the bacterial concentrations as the highest Chl a, Chl b and Car was calculated compared to control (Table 5). Application of neither I nor A+I showed any significant difference in photosynthetic pigments for both the concentrations, except for A+I 10^7.

Effect of PGB_invit on in vitro 7-day old seedling stage inoculation

In contrast to the effect of bacterium on seed stage inoculations, a beneficial influence of all bacterial populations, except A 10^8, was observed in FW of *in vitro* 7-day old inoculated seedlings where the highest FW (96.08 mg) was obtained from A+I inoculation of bacterium at 10^7 concentration (Table 1). Similar effect was witnessed in DW too (Table 2). The only detrimental effect was observed in SL where shortest shoots were observed (0.20 cm), when I bacterium was applied at 10^8, in comparison to the rest (Table 3). In contrast, positive effect of A+I bacterial population at 10^7 CFU/mL was seen in RL where the longest root (8.67 cm) was measured (Table 4, Fig. 1). Similar outcome was seen for Chl a and Chl b with A 10^8 and I 10^7 populations of the bacterium, respectively (Table 5). No significant effect on carotenoids was observed for any bacterial inoculation.

Table 1. Influence of PGB_invit inoculation in different physiological stages on fresh weight (FW) of *A. thaliana* seedlings[a]

Inoculation (CFU/ml)	Germination Stage[b,c] (Mean±SE)[d] (mg)	Seedling Stage[b,c] (Mean±SE)[d] (mg)
Control	43.58±0.73ab	58.00±2.01c
10^7 A[c]	18.08±0.72d	71.67±2.99bc
10^8 A[c]	14.67±0.60d	63.50±1.41c
10^7 I[c]	45.50±0.31a	80.58±1.17b
10^8 I[c]	33.08±0.76c	79.25±2.31b
10^7 A+I[c]	47.08±0.47a	96.08±1.99a
10^8 A+I[c]	40.08±1.45b	80.75±2.17b

[a] The data were collected 7 or 14 days after inoculation for seedling stage and germination stage, respectively. Each trial was made with at least 10 seeds/seedlings and the trials were repeated at least 3 times.
[b] "Germination Stage" means that PBG_invit inoculation was made to surface sterilized seeds whereas "Seedling Stage" means that PBG_invit inoculation was made to 7 day-seedlings following *in vitro* germination.
[c] The different letters following the means show the statistical difference compared to the LSD test following ANOVA (P≤0.05). Difference was evaluated vertically.
[d] Mean±Standard Error; [c] A: active form of bacterium showed; I: inactive form of bacterium; A+I: active and inactive form of the bacterium.

Table 2. Influence of PGB_invit inoculation in different physiological stages on dry weight (DW) of *A. thaliana* seedlings[a]

Inoculation (CFU/ml)	Germination Stage[b,c] (Mean±SE)[d] (mg)	Seedling Stage[b,c] (Mean±SE)[d] (mg)
Control	2.56±0.05a	3.69±0.10c
10^7 A[c]	1.50±0.04c	4.26±0.05b
10^8 A[c]	1.48±0.11c	3.77±0.12c
10^7 I[c]	2.60±0.09a	4.78±0.11ab
10^8 I[c]	2.24±0.04ab	5.84±0.13a
10^7 A+I[c]	2.73±0.03a	5.29±0.12ab
10^8 A+I[c]	2.23±0.05b	6.00±0.10a

[a] The data were collected 7 or 14 days after inoculation for seedling stage and germination stage, respectively. Each trial was made with at least 10 seeds/seedlings and the trials were repeated at least 3 times.
[b] "Germination Stage" means that PBG_invit was inoculated to surface sterilized seeds whereas "Seedling Stage" means that PBG_invit was inoculated to 7 day-seedlings following *in vitro* germination.
[c] The different letters following the means showed the statistical difference compared to the LSD test following ANOVA (P≤0.05). Difference was evaluated vertically.
[d] Mean±Standard Error; [c] A: active form of bacterium; I: inactive form of bacterium; A+I: active and inactive form of the bacterium.

Table 3. Influence of PGB_invit inoculation in different physiological stages on shoot length (SL in cm) of *A. thaliana* seedlings[a]

Inoculation (CFU/ml)	Germination Stage[b,c] (Mean±SE)[d] (mg)	Seedling Stage[b,c] (Mean±SE)[d] (mg)
Control	0.39±0.03c	0.26±0.01a
10^7 A[c]	0.35±0.01c	0.23±0.01ab
10^8 A[c]	0.25±0.01d	0.24±0.01a
10^7 I[c]	0.48±0.02b	0.26±0.00a
10^8 I[c]	0.37±0.00c	0.20±0.00b
10^7 A+I[c]	0.39±0.01c	0.26±0.01a
10^8 A+I[c]	0.61±0.00a	0.23±0.00ab

[a] The data were collected 7 or 14 days after inoculation for seedling stage and germination stage, respectively. Each trial was made with at least 10 seeds/seedlings and the trials were repeated at least 3 times.
[b] "Germination Stage" means that PBG_invit inoculation was made to surface sterilized seeds whereas "Seedling Stage" means that PBG_invit inoculation was made to 7 day-seedlings following *in vitro* germination.
[c] The different letters following the means show the statistical difference compared to the LSD test following ANOVA (P≤0.05). Difference was evaluated vertically.
[d] Mean±Standard Error ; [c] A: active form of bacterium; I: inactive form of bacterium; A+I: active and inactive form of the bacterium.

Table 4. Influence of PGB_invit inoculation in different physiological stages on root length (RL in cm) of *A. thaliana* seedlings[a]

Inoculation (CFU/ml)	Germination Stage[b,c] (Mean±SE)[d] (mg)	Seedling Stage[b,c] (Mean±SE)[d] (mg)
Control	8.19±0.32ab	7.80±0.04b
10^7 A[c]	8.13±0.23a	7.01±0.20c
10^8 A[c]	7.17±0.10b	6.84±0.21c
10^7 I[c]	7.52±0.15b	6.91±0.06c
10^8 I[c]	8.21±0.12a	7.06±0.09c
10^7 A+I[c]	8.13±0.25a	8.67±0.07a
10^8 A+I[c]	8.38±0.04a	7.01±0.11c

[a] The data were collected 7 or 14 days after inoculation for seedling stage and germination stage, respectively. Each trial was made with at least 10 seeds/seedlings and the trials were repeated at least 3 times. [b] "Germination Stage" means that PBG_invit inoculation was made to surface sterilized seeds whereas "Seedling Stage" means that PBG_invit inoculation was made to 7 day-seedlings following *in vitro* germination. [c] The different letters following the means showed the statistical difference compared to the LSD test following ANOVA (P≤0.05). Difference was evaluated vertically. [d] Mean±Standard Error; [c] A: active form of bacterium; I: inactive form of bacterium; A+I: active and inactive form of the bacterium.

Table 5. Influence of PGB_invit inoculation in different physiological stages on photosynthetic pigments of *A. thaliana* seedlings[a]

Inoculation (CFU/ml)	Germination Stage[b,c]			Seedling Stage[b,c]		
	Chl a[d]	Chl b[d]	Car[d]	Chl a[d]	Chl b[d]	Car[d]
	(Mean±SE)[e]			(Mean±SE)[e]		
Control	110.57±9.81b	38.70±3.79b	35.90±2.44b	80.71±2.88b	30.60±3.46b	30.40±2.40ab
10^7 A[f]	202.8±17.25a	81.50±9.93a	60.90±4.67a	42.40±6.47c	15.90±2.4c	17.50±2.01c
10^8 A[f]	204.1±12.44a	85.40±6.68a	61.10±3.24a	117.20±14.99a	40.40±4.90ab	36.80±3.43a
10^7 I[f]	75.05±4.23bc	33.20±1.71b	26.10±0.81b	85.10±13.63ab	43.20±6.39a	25.60±3.73b
10^8 I[f]	88.68±8.68bc	38.40±3.08b	30.00±2.09b	107.24±15.02ab	12.06±4.92c	28.50±4.26ab
10^7 A+I[f]	60.40±5.84c	28.60±1.87b	23.80±1.44b	99.20±15.42ab	30.70±3.07bc	31.20±4.30ab
10^8 A+I[f]	89.80±8.60bc	36.90±3.98b	31.40±2.59b	72.80±7.98b	24.70±2.77c	26.40±2.26bc

[a] The data were collected 7 or 14 days after inoculation for seedling stage and germination stage, respectively. Each trial was made with at least 10 seeds/seedlings and the trials were repeated at least 3 times.
[b] "Germination Stage" means that PBG_invit inoculation was made to surface sterilized seeds whereas "Seedling Stage" means that PBG_invit inoculation was made to 7 day-seedlings following *in vitro* germination.
[c] The different letters following the means showed the statistical difference compared to the LSD test following ANOVA (P≤0.05). Difference was evaluated vertically.
[d] Chl a: Chlorophyll A; Chl b: Chlorophyll B; Car: Caretonoids.
[e] Mean±Standard Error.
[f] A: active form of bacterium; I: inactive form of bacterium; A+I: active and inactive form of the bacterium.

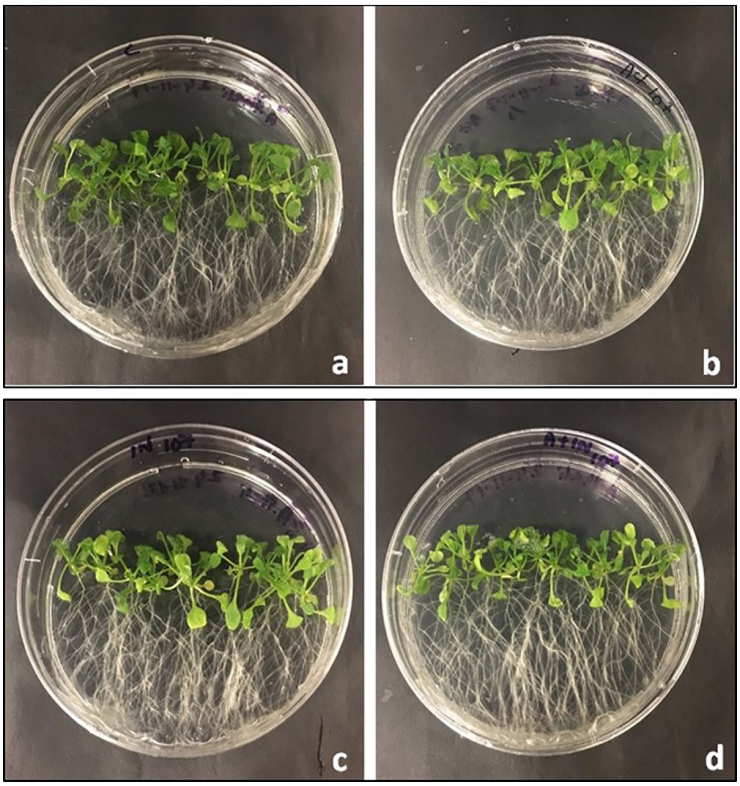

Fig. 1. Phenotypic results of 10^7 inoculation of PGB_invit on 7 day-seedlings of *A. thaliana* after 14 days of culture. a) Control, b) A, c) I and d) A+ I

Molecular confirmation of endophytic and rhizopheric colonization of PGB_invit in seedlings

The MPYE agar Petri plates with experimental inoculants incubated at 30 °C were analyzed for endophytic and rhizospheric colonization of the bacterium. The presence of bacterium was evident in E1 and R1 inoculated plates thus verifying the interaction of PGB_invit with seedlings both endophytically and rhizospherically. As expected, no colony was formed on E2, E3, R2, R3 and sterilization control plates, indicating inactivation of bacteria (E2, R2). Gram staining results from both E1 and R1 single colony staining showed the presence of Gram negative, non-motile bacterial cells existing singly or in pairs, which was in accordance with the characteristics of PGB_invit previously reported by Şeker *et al*. (2017).

Gel Electrophoresis of PCR amplified product obtained with *virD1* universal primers, showed the presence of ~500 bp band from both endophytically and rhizospherically isolated bacterial cultures from the seedlings and from the isolated plasmid DNA of PGB_invit (Fig. 2).

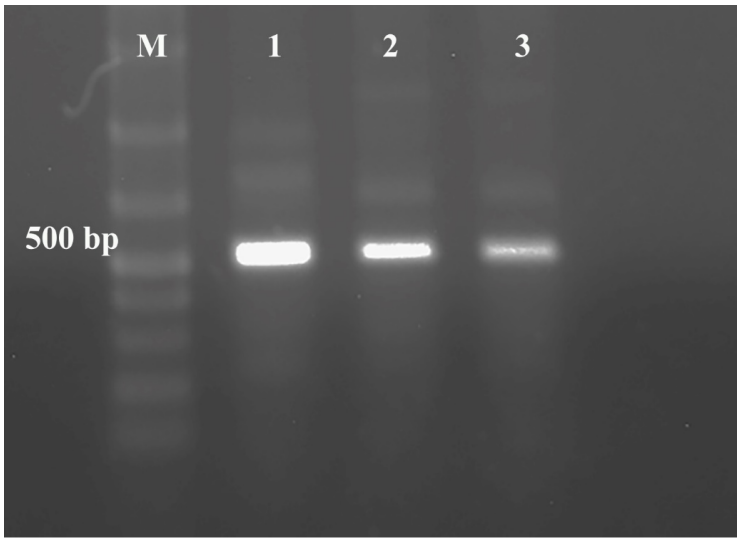

Fig. 2. PCR analysis of plasmid DNA of PGB_invit isolated from endophytic and rhizospheric samples of seedlings. Lane M; 1kb Molecular weight marker, Lane 1; single colony culture, Lane 2; endophytically isolated plasmid DNA, Lane 3; rhizospherically isolated plasmid DNA

Discussion

A wide range of parameters were assessed in this study to reveal out the effect of different concentrations of PGB_invit on the growth and health of *A. thaliana* (Col-0) at two different physiological stages. Our results showed the effectiveness of I form of bacterium together with A+I bacterial compositions on the plant growth. It was interesting to see that I and A+I populations of 10^7 caused an overall increase in FW of the 7-day inoculated seedlings while same results were observed for 10^8 populations in case of DW. While A populations for both concentrations stayed behind for biomass, they positively affected chlorophyll contents of the seedlings.

For seed stage inoculations, I form of the bacterium did not evoke plant defense mechanisms as no decline or decrease in plant growth was observed, while A form alone stayed vulnerable to plant defense systems. Here, it is important to discuss about the difference of effects for two tested concentrations of bacterium populations. For instance, I 10^7 and 10^8 populations remained more or less positively affective towards all the parameters of the seedlings whether alone or co-cultivation with A, while A populations (10^7 and 10^8) kept switching according to the biomass or photosynthetic content ratios and physiological stages of the seedlings.

The positive effects of I form of bacterium can be explained by predicting that cell wall of bacterium was perhaps some kind of hindrance towards direct interaction with plant surface for some of the remaining active cytoplasmic contents, such as certain root and shoot promoting phytohormonal substances. Inactivation might have caused these contents to come in direct contact with the plant surface, thus providing the results perceived. Therefore, it could be stated that I isolate was found positive or even much productive as compared to A for some PGP traits even after subjection to high temperature that partially inactivated the bacterium. High temperature tolerance of possibly growth promoting phytohormonal substances suggests that it can be thinkable to use inactivated form of this putative bacterium with compatible bio-inoculants (bio-fertilizers), but after a series of evaluations for different factors. In a similar kind of study, Nehra and co-workers (2016) evaluated *Brevibacillus brevis* as a potential plant growth promoting rhizobacteria for cotton (*Gossypium hirsutum*) crop by treating with high temperatures (46 °C) and found that bacterium retained its positive activity. Our bacterium partially retained PGP activity as it was subjected to very high temperature (95 °C). Moreover, it even enhanced the positive effect of active bacterium on seedlings thus providing additive support towards growth promotion of plant overall. Therefore, in future, a temperature tolerance studies can be conducted in detail to learn the potential behavior of this bacterium towards PGP activities.

Increase in FW and DW refers to an increased cytokinins and auxins production since these phytohormones are essential for plant growth and development. These results coincide with the earlier findings for this putative endophytic bacterium (Şeker *et al*., 2017). These findings are also in conformity with the literature for PGPRs and endophytes as these are known to produce phytohormones for plant growth (Mendes *et al*., 2007). PGP-invit appeared to be involved in increasing biomass and it is therefore credible to think to use this bacterium with other compatible PGPRs or soil microbes for root biomass and chlorophyll contents in right composition for an overall increase in plant growth. Valdenegro *et al*. (2001) used a wide range of compatible strains including bacteria, fungi and mycorrhizae to enhance the overall growth of *Medicago arborea* in a semi-arid Mediterranean area.

Interestingly, A form of bacterium increased chlorophyll content in seed stage inoculation, which can be related to biomass vs photosynthetic content ratio (Zhang *et al*., 2008). In addition, for seedling stage inoculations, A at 10^8 caused an increase in photosynthetic contents, while less contributing towards seedling biomass. The increase in photosynthetic contents as compared to non-inoculated

seeds can be related to regulatory role of plant symbionts as Zhang *et al*. (2008) suggested that *Bacillus subtilis* GB03 soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels. The accumulation of sugar content in roots or shoots might be considered as the reason of elevated sugar levels induces storage processes and confers feedback inhibition of photosynthesis and vice versa (Jang and Sheen, 1994; Sheen, 1994; Jang *et al*., 1997; Rook *et al*., 2001; Moore *et al*., 2003; Rolland *et al*., 2006). In this regard also, the I form of bacterium studies is very important because of its consistency towards plant growth promotion.

The reason why active populations didn't work well with seed stage inoculation might lies in the fact that seeds maintain their protective state until external environment conditions become favorable for their development process and after imbibition and embryo exposure, emerged seedling become even more sensitive to environmental conditions (Karssen, 1982; Pritchard *et al*., 1993; Bungard *et al*., 1997). While A form of bacterium possibly caused the activation of the seed and later the seedlings plant defense mechanisms, I form might have not activated these defense systems as it was not virtually alive. The same can also be considered true for seedling inoculations.

PCR amplification of plasmid DNA from PGB_invit as a template and using universal forward and reverse primers of *virD1* approved the presence of *virD1* gene on the plasmid of colonies in E1 and R1 plates, suggesting that PGB_invit depicts both rhizospheric and endophytic colonization in *A. thaliana*. In fact, the size of *virD1* gene (500 bp) is slightly different from *A. rhizogenes virD1* gene (~450 bp), (Hamill *et al*., 1991) indicating the fact that the size of *virD1* gene might be slightly longer in PGB_invit.

Conclusions

Overall findings demonstrate that PGB-invit shows both endophytic and rhizospheric colonization in *A. thaliana* and has beneficial impact when inoculated especially at seedling stage in optimum concentrations and conditions. Presence of *virD1* in this bacterium also depicts its possible potential to be used as plant biotechnological studies. However, it is still hard to provide any definitive statement regarding the behavior of PGB_invit because of the complex nature of plant-microbe interactions. The positive influences of PGB_invit previously in fraser photinia and now *A. thaliana* suggests that this bacterium is possibly not host specific. In this regard, study of this bacterium against different potential hosts will be a handy approach to evaluate its potential as a possible PGPR near future. Moreover, the studies at molecular level for *A. thaliana* using molecular tools such as transcriptomics or microarrays will be quite useful to shed light on plant and bacterium interaction.

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Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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