Microbiological Research 172 (2015) 7–15

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

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

Johan Sukweenadhi[a,1], Yeon-Ju Kim[a,*,1], Eul-Su Choi[b], Sung-Cheol Koh[c], Sang-Won Lee[d], Yu-Jin Kim[a], Deok Chun Yang[a,e,*]

[a] Department of Oriental Medicinal Material & Processing, Kyung Hee University, Republic of Korea
[b] GuyeonggiDo Agricultural Research & Extension Services, Gyeonggi-do, Republic of Korea
[c] Department of Environmental Engineering, Korea Maritime and Ocean University, Busan, Republic of Korea
[d] Department of Plant Molecular Systems Biotechnology and Crop Biotech Institute, Kyung Hee University, Republic of Korea
[e] Graduate School of Biotechnology and Ginseng Bank, Kyung Hee University, Republic of Korea

ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form 9 January 2015
Accepted 11 January 2015
Available online 19 January 2015

Keywords:
Plant growth-promoting bacteria
Paenibacillus
Salt stress
Drought stress
Aluminum stress

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

* Corresponding authors at: Department of Oriental Medicinal Material & Processing, Kyung Hee University, Republic of Korea. Tel.: +82 31 201 2100; fax: +82 31 202 2688.
E-mail addresses: yeonjukim@khu.ac.kr (Y.-J. Kim), deokchunyang@khu.ac.kr (D.C. Yang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.micres.2015.01.007
0944-5013/

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