Protection Against Pathogen and Salt Stress by Four Plant Growth-Promoting Rhizobacteria Isolated from *Pinus* sp. on *Arabidopsis thaliana*

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ABSTRACT

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The ability of four plant growth-promoting rhizobacteria, isolated in a previous study, to induce systemic resistance on *Arabidopsis thaliana* Col 0 against biotic and abiotic stress was evaluated. All the bacteria enhanced protection against the foliar pathogen *Pseudomonas syringae* DC3000 and increased plant tolerance to salt stress (NaCl 60 mM). *Bacillus* sp. strain L81 and *Arthrobacter oxidans* strain BB1 performed best with a decrease in the disease index of 61.2 and 52.3%, respectively, and a reduction in the mortality due to salt stress of 72.4 and 57.8%, respectively.

Additionally, significant differences were found in growth and photosynthesis, again, L81 and BB1 performed best either in normal or under stress conditions. In order to elucidate the pathway elicited by these two strains to induce systemic resistance, experiments with the transgenic line of *Arabidopsis thaliana* NahG (defective in salicylic acid [SA]) and with the *jar1* mutant (defective in jasmonic acid) were carried out. Results showed that the SA-dependent pathway was involved in the defense response induced by strains L81 and BB1. Results from quantitative reverse transcription-polymerase chain reaction analysis of the *PR1* gene, related to the SA-dependent pathway and the *PDF1.2* gene related to the SA-independent pathway, showed an increased expression of *PR1* in BB1-treated plants, confirming involvement of the SA-dependent pathway in the defensive response.

Plant growth-promoting rhizobacteria (PGPR) (17) are non-pathogenic beneficial soil rhizobacteria that play a key role in plant health and nutrition (25). These may benefit plant growth, either by improving plant nutrition or producing plant growth regulators (11,13). They may also prevent attack from pathogenic microorganisms (3,41) or help in the establishment of symbiosis with rhizobia or with mycorrhiza (9,45).

PGPR can be found among different bacterial genera such as *Arthrobacter*, *Chryseobacterium*, *Curtobacterium*, *Gluconacetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Herbaspirillum*, and *Pseudomonas* (3,12,26). The mechanisms by which PGPR promote growth have already been widely reported (45), while defensive mechanisms such as induced systemic resistance are currently being intensively investigated from the viewpoint of transduction pathways within plants (8,41).

Plants possess various inducible defense mechanisms to protect themselves against pathogen attacks. A classic example of this is systemic acquired resistance (SAR), which is activated after infection by a necrotizing pathogen. SAR has been demonstrated in many plant species and confers resistance against a broad spectrum of plant pathogens in distant uninfected plant parts (32). Some root colonizing nonpathogenic rhizobacteria may also trigger disease resistance in the host plant, a phenomenon that has been termed induced systemic resistance (ISR) (41). The ISR is a plant mediated mechanism that starts in the roots and extends up to the shoots. It is effective against different types of plant pathogens. The triggering of disease resistance by nonpathogenic bacteria depends on plant species to a different extent. Hence, it is

interesting to find strains that can stimulate a wide array of plants in order to select them for potential commercial uses.

In *Arabidopsis thaliana*, SAR is associated with local and systemic increases in endogenously synthesized salicylic acid (SA) and a coordinated expression of genes encoding pathogenesis-related proteins (PR) (23,39). SA is a necessary intermediate in the SAR signal transduction pathway because SA-nonaccumulating plants (NahG), expressing the bacterial SA hydroxylase gene *NahG*, and other mutants such as SA-induction deficient (Sid) mutants, encoding a mutated isochorismate synthase necessary for the synthesis of SA, are impaired in SAR (14,23). In contrast, there are some bacteria, for example, *Pseudomonas fluorescens* WCS417r, that trigger ISR independent of concomitant PR gene activation (28,29,44,46). Furthermore, there are other metabolic responses that consist in a faster and stronger defense expression, which are only appreciated upon pathogen challenge; this phenomenon is called priming, and has been described for nonpathogenic root-colonizing pseudomonads rhizobacterium-mediated induced systemic resistance (5). The JA response mutant *jar1* and the ethylene response mutant *etr1*, which both express normal levels of pathogen induced SAR (22,23,30), did not express ISR upon treatment with WCS417r, indicating that the ISR-signaling pathway requires components of the JA and ethylene response (18,30). van Wees et al. (44) also demonstrated that the SAR and ISR pathways are fully compatible, resulting in an additive effect on the level of induced protection.

Nowadays special interest is put on research directed to the development of inoculants with beneficial bacteria (PGPR) for environmentally friendly agricultural management. The main purpose of these biofertilizers is stimulation of the plant's defensive metabolism, which will allow crop cultivation in areas with a high pathogen incidence, or on degraded or saline soils or subject to water restriction conditions. Overcoming these issues is a big problem in modern agriculture due to the lack of fertile soils and

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the need to feed an increasing world population. Several authors (4,38) have related the expression of some genes in the defensive response with genes expressed in salt stress situations, e.g., genes involved in ethylene, JA, and abscisic acid pathways. Because of this, plants induced with the proper biological agent may improve their adaptability to salt stress and drought situations (38).

Therefore, our rationale was that PGPR inoculants could improve resistance to pathogens and salt stress in plants. The aim of this work was first, to study the ability of the four PGPR strains to improve growth and to induce systemic resistance in *A. thaliana* Col 0 against biotic (the leaf spot pathogen *P. syringae* DC3000) and abiotic (salt) stress. Secondly, to elucidate whether an SA-dependent or an SA-independent pathway is involved in the response of the most effective strains at a biochemical level, with NahG plants and *jar1* mutants, and at a molecular level, with quantitative RT-PCR analysis of two transcriptional markers, the *PR1* gene, related to the SA-dependent pathway, and the *PDF1.2* gene, related to the SA-independent pathway.

MATERIALS AND METHODS

Bacterial strains. Four PGPR strains isolated from the mycorrhizosphere of *Pinus pinea* L. and *P. pinaster* Aiton and the mycosphere of associated *Lactarius deliciosus* (Fries) S. F. Gray, in a previous study (1), were used: *Staphylococcus* sp. strain I26 (AY307362), *Bacillus* sp. strain L81 (AY307364), *Curtobacterium* sp. strain M84 (AY307369) and *Arthrobacter oxidans* strain BB1 (AY307363, Spanish Type Culture Collection [CECT] 7170). These bacteria showed siderophore and auxin-like compounds production, phosphate solubilization, and ACC degradation abilities (Table 1), and had demonstrated growth promotion effect on *P. pinea* (J. Barriuso, B. Ramos Solano, C. Santamaria, A. Daza, and J. Gutierrez Manero, unpublished data).

The leaf pathogen *Pseudomonas syringae* pv. *tomato* DC3000 was used for challenge inoculation. This strain causes bacterial speck on the model plant *A. thaliana* and is used to study the model system for plant-pathogen interactions (41).

Cultivation of plants. *A. thaliana* seeds ecotype Col 0, transgenic NahG plants harboring the bacterial *NahG* gene (6), and *jar1* mutants (35) were sown in quartz sand watered with Hoagland solution. Two-week-old seedlings were transplanted individually to 100-ml pots. The substrate consisted in a 12:5 (vol/vol) potting soil/sand mixture, autoclaved two times for 20 min each at 121°C, with a 24 h interval between each one. Then, the bacterial inoculum was mixed with the sterilized substrate when appropriate. Seedlings were cultivated in a growth chamber (Sanyo MLR-350H) with a 9-h light (350 $\mu\text{E s}^{-1} \text{m}^{-2}$ at 24°C) and 15-h dark period (20°C) at 70% relative humidity. Plants were watered regularly and supplied with half-strength Hoagland solution (Sigma-Aldrich) once a week throughout the experiment after transplant. All experiments followed a random block design.

Bacterial inoculation. PGPR strains were maintained at -80°C in tryptic soy broth (TSB) amended with 20% glycerol. Inoculum was prepared by streaking strains from -80°C onto plate count agar (PCA, Condalab, Spain) plates, incubating the plates at 28°C for 24 h, and scraping bacterial cells off the plates in sterile

10 mM MgSO_4 buffer. The inoculation process of PGPR was as follows (Fig. 1): sterile substrate was bacterized to achieve 10^8 CFU/g. Therefore, PGPR were applied upon transplant to 2-week-old seedlings as previously described. A second inoculum dose was applied by a soil drench (10^8 CFU/g substrate) 3 weeks after transplant.

P. syringae pv. *tomato* DC3000 was maintained at -80°C in TSB amended with glycerol. Pathogen inoculum was prepared by growing cells in TSB medium overnight, at 28°C under shaking. After centrifugation, bacterial cells were resuspended in 10 mM MgSO_4 buffer, and the concentration was adjusted to 10^7 CFU/ml. Inoculation procedure was as follows: plants were kept at 100% humidity for 24 h to ensure stomatal opening. Leaves of each plant were dipped on a 10^7 CFU/ml solution of *P. syringae* pv. *tomato* DC3000 in 10 mM MgSO_4 buffer and kept in high humidity conditions for the following 24 h. Nonpathogen challenged controls of all treatments were mock-inoculated with 10 mM MgSO_4 buffer and kept in parallel to observe disease progress. In the corresponding treatments, pathogen challenge was carried out 1 week after the second PGPR inoculum, which is 4 weeks after transplant.

Photosynthesis measurement. Photosynthesis was measured in three leaves of each plant in each treatment, with an FMS 2 Fluorescence Monitoring System (Hansatech, Norfolk, UK), under light conditions. Photosynthetic efficiency was measured under light conditions as the efficiency of excitation energy captured by photosystem II reaction centers, estimated by the fluorescence ratio ($F_v/F_m = (F_m - F_0)/F_m$), where F_0 = minimum fluorescence, F_m = maximum fluorescence, F_v = variable fluorescence ($F_m - F_0$), and F_v/F_m = photosynthetic efficiency (10,19,21).

Growth promotion assay and photosynthesis measurement in *A. thaliana* Col 0 plants. *A. thaliana* Col 0 plants were used. A split-plot design was followed. Three groups of plants were defined, with a randomized design within each group. Each group consisted on the four PGPR treatments and a noninoculated control ($n = 12$ in each treatment). One week after the second dose of PGPR, one group of plants was challenged with the pathogen (group 1), another was challenged with NaCl (group 2), and a non-stressed group of plants was left as a control (group 3).

The pathogen group (four PGPR treatments and the control) was inoculated with the pathogen *P. syringae* pv. *tomato* DC3000 as described in previously.

The salt group (four PGPR treatments and the control) was watered with a saline solution to maximum water holding capacity, in order to achieve an electrical conductivity in the substrate equivalent to that of a 60 mM saline solution. Conductivity was determined in the 12:5 (vol/vol) potting soil/sand mixture

Fig. 1. Experimental design for induction of systemic resistance assay inoculation pattern.

TABLE 1. Origin, morphological classification, plant growth-promoting rhizobacteria traits shown in vitro, and 16S rDNA sequence GenBank accession number of tested bacterial strains (1)

Strain	Morphology	Gram	Spore	Origin	Biochemical activity	Accession number
<i>Bacillus</i> sp. L81	Bacilli	+	+	Mycorrhizosphere <i>Pinus pinea</i>	Siderophore production	AY307364
<i>Curtobacterium</i> sp. M84	Bacilli	+	-	Mycosphere <i>P. pinea</i>	Phosphate solubilization	AY307369
<i>Arthrobacter oxidans</i>						
BB1 (former L62)	Bacilli	+	-	Mycorrhizosphere <i>P. pinea</i>	Auxin + siderophore production	AY307363
<i>Staphylococcus</i> sp. I26	Coco	+	-	Mycosphere <i>P. pinaster</i>	Siderophore production + phosphate solubilization + ACC degradation	AY307362

paste, with a conductivity meter Crison (Alella, Spain). The calibration curve for substrate conductivity was obtained with increasing concentrations of NaCl aqueous solutions; conductivity in a 60 mM saline solution was also determined. All measurements were done in triplicate.

One week after stress challenge, shoot fresh weight was measured in all plants and photosynthesis was measured in three leaves of each plant as described in the previously.

Induction of systemic resistance by PGPR upon pathogen challenge and salt stress. The experiment was repeated as described in the growth promotion assay with the difference that in this experiment, 50% of the plants in the pathogen group (four PGPR treatments and the control) were inoculated with the pathogen *P. syringae* pv. tomato DC3000 as described previously. Likewise, 50% of the plants in the salt group were watered with the saline solution as described in the growth promotion assay. One week after stress challenge, disease incidence and salt stress symptoms were assessed by determining the percentage of leaves with disease symptoms (chlorotic spots or wilted leaves) per plant, in stressed and in control plants.

Determination of the induction pathway: transgenic and mutant plants. The experiment of systemic resistance against the leaf pathogen *P. syringae* DC3000 was repeated with the most effective PGPR in protection to biotic and abiotic stress. BB1 and L81 were inoculated, as described previously, in *A. thaliana* Col 0 plants, NahG plants, and *jar1* mutants, following the design shown in Figure 1. In the first assay, plants were pathogen challenged and in a second assay, *A. thaliana* Col 0 plants, NahG plants, and *jar1* mutants were salt stressed as described previously.

Fig. 2. Fresh weight (g) of 6-week old *Arabidopsis thaliana* Col 0 plants inoculated with the four plant growth-promoting rhizobacteria. **A**, Non-stressed, **B**, with biotic stress (leaf spot pathogen *Pseudomonas syringae* DC3000), and **C**, with abiotic stress (60 mM NaCl). Bars indicate standard errors and different letters indicate the existence of significant differences according to Tuckey's test ($P < 0.05$).

ously. In both cases a group of non-stressed plants was kept as control. Eighteen replicates in each treatment were used to elucidate the transduction pathway involved in the defensive response of the plants. Disease incidence was determined 1 week after stress challenge.

Determination of the induction pathway: transcriptional markers. Expression of transcriptional markers of each transduction pathway were measured in *A. thaliana* Col 0 plants, induced with BB1, with L81, and in non-induced controls, 24 h after the last PGPR bacterization (experimental design described previously). Samples were taken as follows: RNA from *A. thaliana* leaves (three replicates made of nine leaves taken from different plants) was extracted with an RNeasy Plant Mini Kit (Qiagen, CA). Five-hundred nanograms of RNA free of DNA and RNases were retrotranscribed in a 20- μ l reaction: 1 $\times$ RT-PCR buffer, 2.5 mM MgCl₂, 1 mM dNTP Blend, 10 U of RNase inhibitor, 1.25 μ M Random Hexamer, and 15 U of MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, CA). The reaction was incubated for 10 min at 25°C and 12 min at 42°C in a GeneAmp 2700 thermocycler (Applied Biosystems).

Five microliters of the retrotranscription reaction was used as a template for real time PCR with specific primers for the *ACTIN1* as the housekeeping gene (ACT[PCRQ]F 5'-CGTTGCACCAC-CTGAAAGG-3' and ACT[PCRQ]R 5'-GGGAAGCAAGAAT-GGAACCA-3'), and for the transcriptional markers *PR1* (PR-1qF 5'-GTCTCCGCCGTGAACATGT-3' and PR-1qR 5'-CGTGTTC-GCAGCGTAGTTGT-3'), and *PDF1.2* (Pdf1.2qnew2F 5'-TTGT-TCTCTTTGCTGCTTTCGA-3' and Pdf1.2qnew2R 5'-TTGGC-TTCTCGCACAACTTCT-3'). The reaction was performed as follows: 300 nM each primer, 1 $\times$ SYBR Green PCR Master Mix (Applied Biosystems) in a 20- μ l reaction. PCR conditions were 40 cycles, with an annealing temperature of 60°C in an ABI Prism 7700 system for quantitative PCR (Applied Biosystems).

A standard curve was performed for each primer with four serial dilutions, and data were normalized with the housekeeping gene expression. The measures were carried out with three replicates formed with nine plants in each one and the experiment was repeated twice with similar results.

Statistical analysis. To evaluate bacterial effect on growth, photosynthesis and decrease of stress incidence, unidirectional analysis of variance (ANOVA) were carried out. When differences were significant, a Tukey test ($P < 0.05$) was performed with the computer program Statgraphics plus 5.1, for Windows.

RESULTS

Growth promotion assay and photosynthesis measurement in *A. thaliana* Col 0 plants. The four PGPR bacterial strains *Staphylococcus* sp. strain I26, *Bacillus* sp. strain L81, *Curto-bacterium* sp. strain M84 and *Arthrobacter oxidans* strain BB1 were tested in *A. thaliana* for their ability to enhance plant growth in normal or under stress conditions (Fig. 2A, B, and C). Only BB1 treated plants showed significant increases in fresh weight in nonstressed plants (Fig. 2A). Pathogen challenged plants inoculated with M84 and BB1 showed significantly higher values of fresh weight than the controls (Fig. 2B) while in salt stressed plants, only those inoculated with BB1 and L81 showed significantly higher values (Fig. 2C). It should be pointed out that noninoculated stressed plants showed lower weight values than nonstressed plants.

Photosynthetic values in nonstressed plants were only significantly enhanced by strain L81 (Fig. 3A). All PGPR-inoculated pathogen-stressed plants showed significantly higher values than the pathogen control (Fig. 3B); also, these values were similar to those registered in PGPR-inoculated nonstressed plants (Fig. 3A and B). On salt-stressed plants (Fig. 3C), photosynthesis values were lower than nonstressed or pathogen-stressed plants. However, salt-stressed plants treated with strains I26, BB1, and L81

showed significantly higher values than the salt-stressed control plants.

Induction of systemic resistance by PGPR upon pathogen challenge and salt stress. Regarding the induction of systemic resistance in *A. thaliana* Col 0 plants, all strains reduced the disease index with statistical significance ($P < 0.05$) against the leaf pathogen *P. syringae* DC3000 (Fig. 4A), with reductions in the disease index of 61.2, 58, and 52.3% achieved by strains L81, M84, and BB1, respectively.

With regard to the salt stress protection in *A. thaliana* Col 0 plants (Fig. 4B), all strains significantly reduced chlorotic symptoms ($P < 0.05$), except for strain M84 (Fig. 4B), being the reductions in the disease index due to L81 and BB1 72.4 and 57.8%, respectively.

Determination of the induction pathway: transgenic and mutant plants. Following the initial evaluation, the two strains which had performed best in protection against biotic and abiotic stress (L81 and BB1) were studied in order to elucidate the pathway elicited by each to induce systemic resistance by the means of transgenic and mutant plants. In this assay, strains L81 and BB1 significantly reduced disease symptoms in pathogen challenged (54.4 and 43.6%, respectively) (Fig. 5) and in salt stressed *jar1* mutants (36.6 and 38.5%, respectively) (Fig. 6), compared with the non-PGPR *jar1* mutants controls. However, NahG plants treated with L81 or BB1 showed higher disease index when challenged with the foliar leaf pathogen DC3000 (110 and 167%, respectively). On the other hand, neither BB1- or L81-treated NahG plants showed any significant protection against salt stress (Fig. 6). Therefore, neither of the two strains was capable of inducing systemic resistance against biotic or abiotic stress in the NahG transgenic line. The increased disease index on transgenic NahG plants is striking (Fig. 5).

Fig. 3. Photosynthesis ($F_v/F_m = (F_m - F_0)/F_m$) on 6-week-old *Arabidopsis thaliana* Col 0 plants inoculated with the four plant growth-promoting rhizobacteria. **A**, Nonstressed, **B**, with biotic stress (leaf spot pathogen *Pseudomonas syringae* DC3000), and **C**, with abiotic stress (60 mM NaCl). Bars indicate standard errors and different letters indicate the existence of significant differences according to Tukey's test ($P < 0.05$).

Determination of the induction pathway: transcriptional markers. In the study of transcriptional markers, the expression of two genes (*PR1* and *PDF1.2*) was measured in PGPR-induced plants and in noninduced controls. *PDF1.2* was expressed at the same level in controls and in BB1- and L81-inoculated plants

Fig. 4. Relative disease index in 6-week-old *Arabidopsis thaliana* Col 0 plants inoculated with the four plant growth-promoting rhizobacteria and the noninoculated control, 1 week after challenge with **A**, the leaf spot pathogen *Pseudomonas syringae* DC3000 and **B**, 60 mM NaCl; the control is expressed as 100% of disease incidence (disease index for control DC3000-treated plants = 50.12%, disease index for control salt-stressed plants = 69.93%). Different letters indicate the existence of significant differences according to Tukey's test ($P < 0.05$), performed separately for the pathogen-challenged and the salt-stressed plants.

Fig. 5. Relative disease index in 6-week-old *Arabidopsis thaliana* Col 0, NahG transgenic line and *jar1* mutants inoculated with the two plant growth-promoting rhizobacteria strains, BB1 and L81, and noninoculated control, 1 week after challenge with the leaf spot pathogen *Pseudomonas syringae* DC3000; the control expressed as 100% of disease incidence (disease index for control DC3000-treated plants = 45.06%, disease index for NahG DC3000-treated plants = 37.27%, and disease index for *jar1* DC3000-treated plants = 42.72%). Different letters indicate the existence of significant differences according to Tukey's test ($P < 0.05$), performed separately for wild type, NahG, and *jar1* plants.

(Fig. 7) while *PR1* expression level in BB1-treated plants was 1.84 times the controls. There was no overexpression in L81-treated plants (0.74 times the controls).

DISCUSSION

Search for specialized PGPR to develop inoculants to trigger defensive metabolism in plants is a challenge to improve productivity in intensive production with high pathogen incidence and in low fertility soils. In this study, four PGPR isolated in a previous work (1) were tested for their ability to promote plant growth and induce systemic resistance on *A. thaliana* against biotic stress (the leaf spot pathogen *P. syringae* DC3000) and abiotic stress (salt). These strains had shown putative PGPR activities in vitro (Table 1), and hence appeared as good candidates to induce systemic resistance.

Although all strains were able to promote growth in *P. pinea* seedlings (Barriuso et al., unpublished data) and all have shown PGPR traits in vitro (1), only BB1 significantly enhanced *A. thaliana* Col 0 growth (Fig. 2A). This may be attributed to the fact that most of the strains showed putative PGPR abilities related to plant nutrition and therefore, these strains would only be effective under nutrient stress conditions (31), which is not the case. Consistent with this argument is the ability of BB1 to release auxin-like compounds that may explain its better performance (31,33). However, when PGPR-treated plants were stressed, either with biotic (the foliar pathogen *P. syringae* DC3000) or abiotic (salt) stress (Fig. 2B and C), higher weight values were found as compared to their controls; BB1- and M84-induced plants performed best under biotic stress conditions, and BB1- and L81-induced plants under abiotic stress conditions. That is, PGPR had a priming effect on plants that is revealed only upon stress challenge (5). This is consistent with data from fresh weight in which PGPR-induced plants perform better than nontreated controls, either under stress or normal conditions.

The photosynthesis value is highly related to the stress level in a plant; salt stress in the roots triggers the expression of abscisic acid pathway genes (38) resulting in increased abscisic acid levels, which is in turn translocated to the leaves, where it closes the stomata, thereby decreasing the rate of photosynthesis (21). Hence, the four PGPR strains assayed improved plant health to a different extent depending on the assayed conditions, revealing different underlying mechanisms for each PGPR as indicated by the two parameters evaluated: fresh weight and photosynthesis. BB1 and L81 performed best.

Fig. 6. Relative disease index in 6-week-old *Arabidopsis thaliana* Col 0, NahG transgenic line and *jar1* mutants inoculated with the two plant growth-promoting rhizobacteria strains, BB1 and L81, and noninoculated control, 1 week after challenge with 60 mM NaCl; the control expressed as 100% of disease incidence (disease index for control salt-stressed plants = 68.31%, disease index for NahG NaCl-treated plants = 75.65%, disease index for *jar1* NaCl-treated plants = 8.71%). Different letters indicate the existence of significant differences according to Tukey's test ($P < 0.05$), performed separately for wild type, NahG, and *jar1* plants.

The pathogen challenge experiment demonstrated that the induction of resistance in the plant is systemic, as PGPR are applied onto the roots and the response is detected in the shoot system, where the pathogen is applied. Interestingly, all strains that induced systemic resistance were gram positive, when this triggering capacity is usually attributed to gram negative strains because of their cell wall composition (47). However, induction of systemic resistance by gram positive strains has been previously described (8,16).

Regarding the induction of systemic resistance in *A. thaliana* Col 0 plants against the leaf pathogen *P. syringae* DC3000, our results show that all strains assayed significantly decreased disease index when compared to the control. Also, all strains protected plants against salt stress except for strain M84 that protects but not significantly (Fig. 4). Therefore, all of them had primed plants as confirmed by the increased protection to pathogen and salt stress, confirming that priming is shown only upon stress challenge. It is striking that 3 of the 4 strains BB1, L81, and I26 were able to palliate both disease and salt stress symptoms. Although, several authors have described that the mechanisms by which PGPR diminish disease index and palliate salinity symptoms may be common, since in the process of induction of systemic resistance the coordinated activation of certain genes occurs (4,38), this needs to be confirmed for each bacteria as revealed by our results in the case of M84, that protected only against biotic stress. Some of the induced proteins protect against plant pathogens (pathogenesis related proteins) (42), and others against salt stress (genes related to oxidative stress, such as cytochrome P450, glutathione reductase) (20).

It is remarkable that strain M84 was able to induce systemic resistance against the leaf pathogen *P. syringae* DC3000, while unable to protect against salt stress. This fact indicates that different pathways can be triggered upon induction, involving different sets of genes being effective against different biotic or abiotic stresses. Many authors have described the possibility that a plant's defense efficiency against a determined pathogen depends on the kind of mechanism used by the plant, and the nature of the elicitor is a key factor upon elicitation. It has been previously demonstrated with *Arabidopsis* genotypes affected in their responses to SA, JA, or ethylene (ET), that basal resistance against *Hyalopenospora parasitica*, Turnip crinkle virus and *P. syringae* is predominantly conferred by SA-dependent defense responses (6,15,29), whereas basal resistance against *Alternaria brassicicola* is mainly controlled by JA, and to a lesser extent by ET (27,36,37).

The second objective of this work was to determine if the mechanism to induce systemic resistance of BB1 and L81 involves the SA-dependent or SA-independent pathway. Strains BB1 and L81 significantly reduced disease index against *P. syringae* DC3000 in wild type plants (42.3 and 34.8%, respectively) and in *jar1* mutants, (54.4 and 43.6%, respectively) (Fig. 5). The same

Fig. 7. Gene expression levels of the transcriptional markers *PR1* (SA-dependent pathway) and *PDF1.2* (SA-independent pathway) in *Arabidopsis thaliana* Col 0 plants, inoculated with the two plant growth-promoting rhizobacteria strains, BB1 and L81, and the noninoculated control.

results were observed against salt stress; BB1 and L81 treated plants showed a significant mortality reduction in wild type plants (59.3 and 54.7%, respectively) and in *jar1* mutants (36.6 and 38.5%, respectively) (Fig. 6). However, in NahG plants, these strains did not cause any effect against biotic or abiotic stress and disease index was even higher than the non-PGPR-inoculated Col 0 control, probably due to the higher energy cost of the induction. BB1 induced plants may be experiencing a “detour” of energetic resources to defensive pathways. Since the subsequent signal transduction is not effective because the plant lacks the transduction signal (SA), their health is compromised when compared to noninduced plants. The same situation is evidenced for L81 but not as intense (40). Therefore, the response against biotic and abiotic stress triggered by BB1 and L81 was SA-dependent. In the case of BB1 the higher expression of PR1 gene compared to that of the control plants supports this notion (Fig. 7) and is consistent with other authors that report PGPR-induction by SA-dependent pathway and PR activation (8,42). However, the increase in PR1 expression was not detected in L81 induced plants. This could be due to several reasons, among them are, (i) the moment of induction, samples for RNA were collected 24 h after the second PGPR inoculation, a time point when this gene may not be induced by this PGPR, (ii) the unsuitability of the transcriptional marker (any pathogenesis related protein of the family (PR3, PR5) could have another induction sequence and serve as a marker at this moment). In this sense, since PGPR are different, a different time course induction cannot be discarded. Irrespective of the hypothesis stated above, De Meyer et al. (7) demonstrated the induction of SA-dependent systemic resistance by *Pseudomonas aeruginosa* in tobacco in absence of detectable PR1 expression, which support data from L81.

Data presented in this study (similar expression levels of the SA-independent route transcriptional marker [*PDF1.2*], and the independence of JA for the induction of systemic resistance, as indicated by *jar1* mutants) support the notion that BB1 and L81 do not use the ET-JA transduction pathway to induce systemic resistance. On the other hand, the enhanced expression levels of PR1 in BB1-treated plants indicate that the SA-dependent route is necessary for induction of systemic resistance as suggested by other authors (42).

It may be concluded that the assayed strains are able to trigger *A. thaliana* systemic resistance which is effective against biotic (pathogen DC3000) and abiotic (salt) stress. In addition, it is shown that the SA-dependent pathway is involved in the response triggered by BB1. Irrespective of the pathway involved, these strains are putative PGPR to be used in intensive agricultural production processes to decrease chemical inputs, and increase crop yield in saline soils, although this capacity needs to be proven first in field trials.

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