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The Saharan isolate *Saccharothrix algeriensis* NRRL B-24137 induces systemic resistance in *Arabidopsis thaliana* seedlings against *Botrytis cinerea*

Saima Muzammil · Clotilde Graillon · Rayenne Saria ·
Florence Mathieu · Ahmed Lebrihi · Stéphane Compant

Abstract

Background and aim *Saccharothrix algeriensis* NRRL B-24137, isolated from a Saharan soil, has been described as a potential biocontrol agent against *Botrytis cinerea* and other phytopathogens. However, the plant protection mechanisms involved still need to be described. The aim of this study was to determine this protection phenomenon as well as parts of the mechanisms involved, using *Arabidopsis thaliana* seedlings and *B. cinerea*.

Methods The bacterial colonization process was evaluated on *A. thaliana* seedlings using fluorescence in situ hybridization. Protection of *A. thaliana* seedlings inoculated with NRRL B-24137 against *B. cinerea* was then evaluated. Parts of the mechanisms involved in the systemic protection against *B. cinerea* were

evaluated using known mutants of genes involved in jasmonate (JA)/ethylene (ET)/salicylic acid (SA) signaling. Other *Arabidopsis* mutants, *AtrbohD-3*, *AtrbohF-3*, and *ups1-1* were also screened to determine other parts of the mechanisms involved.

Results The results showed that the strain NRRL B-24137 colonized, epi- and endophytically, the roots of *Arabidopsis* seedlings but the strain was not a systemic colonizer during the time of the experiment. The strain NRRL B-24137 also reduced *B. cinerea* symptoms and the protection was linked to known mechanisms of induced systemic resistance (ISR; JA/ET signaling), as well as to functionality of AtrbohF oxidase and of UPS1. Crosstalk between ET/JA and SA signaling could also be involved.

Conclusions The isolate NRRL B-24137, after colonizing the root systems of *A. thaliana*, induces an ISR against *B. cinerea*, which is JA/ET dependent, but could also require SA crosstalk and protection could also require NADPH oxidases and UPS1 functionalities.

Keywords *Arabidopsis* · ISR · *Saccharothrix algeriensis* NRRL B-24137 · Soil · Endophyte

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Introduction

Plant growth-promoting rhizobacteria are known to help their hosts by increasing plant growth through changes in nutrient status and phytohormones, and by reducing phytopathogenic infections directly via biocontrol mechanisms or indirectly with induction of a

plant systemic resistance (Bakker et al. 2007; Lugtenberg and Kamilova 2009; Zamioudis and Pieterse 2012). A subset of the rhizosphere microflora can also colonize plants, establish endophytic subpopulations, and spread to various plant parts and tissues (Rosenblueth and Martínez-Romero 2006; Hallmann and Berg 2007; Reinhold-Hurek and Hurek 2011). However, there is still a gap in our knowledge regarding which specific soil bacteria can induce systemic resistance against phytopathogens and if they can be endophytic following rhizosphere colonization (Compant et al. 2005; Compant et al. 2010a; Reinhold-Hurek and Hurek 2011). Such interactions have been demonstrated for some bacterial taxa. For others, this information remains unknown.

Research of new biocontrol agents as well as endophytic colonizers can be relevant for agriculture. Several new sources of biocontrol agents could be exploited and could lead to the discovery of new strains and/or new taxa to help plants. Among these sources, there is a current interest on microbes from desert soils or from plants growing on these soils, and on their use in protecting plants against diseases (Compant et al. 2010b). Harsh environments, such as desert soils and plants growing on these soils, might provide a rich source of beneficial bacteria. It is becoming increasingly evident that these microbes may represent an enormous and untapped genetic reservoir for plant improvement (Barrow et al. 2008) and could act as biocontrol agents against several phytopathogens. These kinds of microbes have, however, not been well exploited for agriculture. Recent evidence has shown that some of these microbes isolated from this kind of environment can help nonhost plants against abiotic and biotic stresses, and additional studies related to the use of such microbes could therefore be of interest for agriculture.

One of the microbes isolated from a harsh environment could be an actinomycete member of the *Actinosynnemataceae* family, which was isolated from soil in a palm grove of Adrar (Algeria). The strain was identified as *Saccharothrix algeriensis* NRRL B-24137 (Zitouni 1995; Zitouni et al. 2004). The strain NRRL B-24137 is known to secrete various secondary metabolites, such as dithiolopyrrolones, which could have broad bioactive activities (Lamari et al. 2002a, b; Zitouni et al. 2005). The strain can also act as a biocontrol agent. It can reduce the growth of *Botrytis cinerea* and other phytopathogens directly (Lamari

2006). On grapevine plants, when the plants have been inoculated with NRRL B-24137, gray mould symptoms due to *B. cinerea* are reduced. This has been demonstrated at 25 °C and high temperature under greenhouse conditions (Muzammil et al. 2011). However, it remains unknown how the bacterium can protect the plants against *B. cinerea*, if the bacteria can induce a state of systemic resistance against phytopathogens or if the protection is linked to an interaction between the biocontrol strain and the necrotroph *B. cinerea* in the aerial plant parts.

Some beneficial rhizo- and/or endophytic bacteria can reduce pathogenic infections directly by niche exclusions, secretion of antibiotics, and reduction of pathogen signaling (Lugtenberg and Kamilova 2009; Mitter et al. 2013). Depending of the strains and on the plants with which they interact, they can also induce plant resistance, which can be correlated to a form of induced systemic resistance (ISR), while pathogenic infections are linked to systemic acquired resistance (SAR). Several strains belonging to different bacterial genera were shown to induce ISR against pathogens, such as some members of fungi, *Peronosporomycetes*, bacteria and insects, as well as viruses, depending of the pathosystems used (van Loon et al. 1998; Lugtenberg and Kamilova 2009; Pineda et al. 2010). This has been well studied, for instance with some *Bacillus* spp., *Pseudomonas* spp., and *Actinobacteria* members but also with other kinds of genera (Mitter et al. 2013). However for some specific microbes, information is still missing.

Extensive research has shown that beneficial microbe-mediated ISR is controlled by signaling pathways, in which ethylene (ET) and jasmonate (JA) play a key role (van Loon et al. 1998; van Wees et al. 2008; van der Ent et al. 2009; Pieterse et al. 2012), whereas salicylic acid (SA) does not (Pieterse et al. 1996; Pieterse and van Loon 1999). In some plant–beneficial bacteria interactions, JA/ET signaling pathways are however not involved but the phenomenon of resistance requires SA signaling (Maurhofer et al. 1994, 1998; Audenaert et al. 2002; Barriuso et al. 2008; van de Mortel et al. 2012), and the intimate association between plants and the beneficial bacteria leads to PR gene expression (such as genes involved in PR-1, PR-2, and PR-5 synthesis). Different mechanisms involved in resistance induced by beneficial microbes have been demonstrated, depending on beneficial strain identity, as well as on the pathosystems used. This has been

widely described using *Arabidopsis* and other plant systems.

Using various mutants of *Arabidopsis thaliana* (*ein2*, *ein4*, and *ein5-1* for ET-dependent pathway and with *jar1* and *allene oxide synthase (aos)* for the JA pathway; van der Ent et al. 2009), mechanisms of ET/JA signaling in ISR have been described. Various gene products have been correlated to ET/JA pathway, such as some enhanced disease susceptibility (*eds*) gene products that can be required for ISR (*eds4* and *eds8*, ET and JA signaling dependent, respectively), whereas SA signaling has been linked to *eds5* products (Ton et al. 2002). The node of convergence NPR1 (NON EXPRESSOR OF PR PROTEINS) was also shown to be associated with ISR (JA/ET dependent), and also with SA signaling (Pieterse and van Loon 2004). Some phytoalexin products were additionally demonstrated as required for ISR and are JA-dependent (PAD1) signaling, while others were demonstrated as involved in SA signaling (PAD3 and PAD4; Glazebrook et al. 2003; Zhou et al. 1998).

The study of new putative mechanisms that could be involved in resistance induced by beneficial microbes could be of special interest for plant–microbe association studies. Some events were described, for instance, as associated with SAR mechanisms, whereas it is still unknown if they can be involved in ISR (JA/ET or SA dependent's phenomena). Under pathogenic infections, NADPH oxidase respiratory burst oxidase homolog D (RBOHD) and respiratory burst oxidase homolog F (RBOHF; Torres and Dangel 2005) are linked to radical oxygen species (ROS) production. Interestingly, UNDERINDUCER AFTER PATHOGEN AND STRESS1 (UPS1) was also demonstrated to be involved in stress signaling following plant perception of pathogenic or abiotic stresses (Denby et al. 2005). It is, however, unknown if UPS1 is required for the resistance induced by beneficial microbes and against plant pathogens. Mutants of Camalexin *ups1* have reduced expression of phosphoribosylanthranilate transferase, a tryptophan biosynthetic enzyme. This mutant can be defective in a wide range of defense responses due to a reduction in SA and JA/ET pathway signaling. Denby et al. (2005) also demonstrated that this mutant is compromised in the expression of reduced ROS genes. UPS1 could be involved in ISR mechanisms, although this still needs to be demonstrated.

In this study, the interaction between strain *S. algeriensis* NRRL B-24137 and *A. thaliana* plants

was partly evaluated. This was performed in order to ascertain if the strain can, following bacterial inoculation and colonization, induce an ISR phenomenon against *B. cinerea*, with JA/ET- or SA-dependent mechanisms. Various plant mutants with reduced JA/ET or SA signaling were used. Other mutants, *AtrbohD-3*, *F-3*, and *ups1-1*, were additionally screened to monitor if the gene products could be involved in the resistance induced by NRRL B-24137 against *B. cinerea*. As the strain was isolated from an underexplored environment for plant improvement, the impact of the strain on plant resistance could be of special interest, in understanding the plant–microbe interaction and to determine which kinds of mechanisms could be involved.

Materials and methods

Bacterial and fungal culture

S. algeriensis NRRL B-24137 was used throughout this work. This strain was grown at 30 °C on International *Streptomyces* Project 2 (ISP 2) solid medium (pH 7.0) containing per liter of distilled water: 4 g D(+) glucose (Acros organics, France), 10 g malt extract (Fluka, France), 4 g yeast extract (Fluka, France), and 18 g agar (Sigma-Aldrich, France). Eight days after growing on plate, aerial mycelium + spores of strain NRRL B-24137 were harvested in PBS and concentration was adjusted to approximately 5×10^7 CFU mL⁻¹.

B. cinerea strain BC1 (isolated from grapevine plants in 2008 in Austria) was grown on potato dextrose agar (Sigma-Aldrich, France) medium at 25 °C under light conditions. Spores of *B. cinerea* were harvested from a 9-day-old culture and concentration was then adjusted with a Thoma cell at 6.5×10^5 conidia mL⁻¹ in a half potato dextrose broth (Sigma-Aldrich, France) solution.

Plant material

Seeds of *A. thaliana* accession Columbia (Col parent accession number N39005 and Col-0 N1092) and mutants *eds4-1* (N3799), *eds5-1* (N3735), *eds8* (N3800), *eds9-1* (N3736), *ein2-1* (N8844), *ein4* (N8053), *ein5-1* (N8054), *jar1-1* (N8072), *col-6(gl-1)* (N8155), *aos* (N6149), *npr1-1* (N3726), *npr1-3* (N3802), *pad1* (N3803), *pad3-1* (N3805), *pad4-1* (N3806), *AtrbohD-3* (N9555), *AtrbohF-3* (N9557), *AtrbohDF-*

3 (N9558), and *ups1-1* (N-6582) were obtained from the Nottingham *Arabidopsis* stock center (NASC).

Seeds of *Arabidopsis* were surface sterilized with 70 % ethanol (5 min), 2.6 % NaClO (2 min), and then rinsed three times with sterilized distilled water. Seeds were, then, placed on plates containing Murashige and Skoog medium (Sigma, France), amended with 2 % saccharose and 0.8 % agar (pH 5.7). Seedlings were allowed to grow for 16 days in a growth phytotronic chamber (16 h photoperiod, 20–25 °C night–day, and 70 % relative humidity) on vertically placed plates.

Induction treatment

Sixteen-day-old WT or mutant seedlings were taken from in vitro plates, rinsed with sterilized distilled water, and the roots of seedlings were dipped in a suspension of the strain NRRL B-24137 or with PBS (control) for 3 min. The seedlings were planted in plates perforated at their bottom and amended with twice sterilized (autoclaved) soil (containing per volume 1/3 perlite, 1/3 sand, 1/3 potting soil). The seedlings were allowed to grow horizontally for 10 days in a box covered with a transparent perforated film and sterilized tap water was added daily with a syringe.

Bacterial populations on and inside seedlings

Roots and leaves of WT seedlings (Col N39005) were sampled 0, 1, 3, 5, 7, and 10 days post-NRRL B-24137 inoculation, in order to evaluate bacterial populations on and inside plant tissues of WT seedlings. Similar experiments were carried out 72 h post-inoculation with *B. cinerea*.

For bacterial root populations, roots were washed with distilled water and 100 mg samples used. To evaluate endophytism of strain NRRL B-24137, roots and leaves were washed and were then surface sterilized using ethanol 70 % (5 min), 2.5 % bleach (1 min), and rinsed three times with sterile distilled water. One hundred milligrams of each plant tissue was then ground with a mortar and pestle and 1 mL of distilled water was added to the samples. The solution was then serially tenfold diluted in PBS and 100 µL of each dilution was plated on a plate with ISP 2 solid medium and cycloheximide (30 mg/mL). Microbes on plates were allowed to grow for 1 week to determine the presence or absence of NRRL B-24137 on the samples.

FISH microscopy

Plant tissues from seedlings WT (Col parent N39005), 10 days post-inoculation with strain NRRL B-24137, were fixed overnight at 4 °C in a paraformaldehyde solution (4 % in PBS), and then rinsed twice with PBS. The samples were dehydrated in an ethanol series (50 to 99.9 %; 30 min each step) and parts of them were embedded in LR white resin according to the manufacturer instructions (Sigma-Aldrich, Germany). Embedded tissues were sliced with a microtome and glass knives, and slices of 1–1.5 µm were deposited on microscopic slides. Fluorescence in situ hybridization was then carried out on slices (for endophytism) or non-embedded tissues (for surface colonization) using each time 10 to 20 µL of a 10 ng µL⁻¹ probe concentration according to Compant et al. (2011) using a mixture of EUB338 (Amann et al. 1990), EUB338II, EUB338III (Daims et al. 1999) coupled with a DyLight 488 dye (Genecust, Luxembourg), and then a HGC69a probe (Roller et al. 1994; corresponding to *Actinobacteria*) coupled with a DyLight 594 dye (probes were bought at Genecust, Luxembourg and dyes at Thermo-Fisher, France). Slices or non-embedded samples without fluorescence in situ hybridization (FISH) hybridization were used as controls. Plants without strain NRRL B-24137 inoculation (PBS) were also used as controls. The samples were then kept in the dark during at least 1 day for air drying. The samples were observed under an epifluorescence microscope (BH2, Olympus, Japan) under an UV light, and pictures were taken with a camera (TCC-3.3ICE-N, Tucsen, China).

Challenge inoculation and disease assessment

Five leaves of each *A. thaliana* seedling (WT or mutants), inoculated with or without NRRL B-24137, were infected 10 days post-bacterial inoculation with a solution of 9 days old *B. cinerea* culture by inoculation with 3 µL of 6.5×10⁵ conidia mL⁻¹. To ensure infection, the inoculated plants were kept at 100 % relative humidity during the entire infection process in sealed boxes (with a transparent film), under phytotronic chamber conditions as described before.

Three days after the challenge, infection caused by *B. cinerea* on seedlings was evaluated by measuring the surface area of necrosis per leaf of *Arabidopsis* seedlings with the help of image J software.

Statistical analyses

All experiments were repeated three independent times with similar results on each of the ten plants. Statistical analyses were performed using min–max normalization (Hann and Kamber 2000) before using the Student's *t* test (<http://studentsttest.com/>).

Results

S. algeriensis NRRL B-24137 colonizes roots of *A. thaliana* but is not present inside leaves that can be infected by *B. cinerea*

The colonization of *A. thaliana* seedlings by the strain NRRL B-24137 was evaluated using FISH analysis or by dilution plating assay of crushed samples using WT Col N39005 seedlings. The bacterium was found on the surface of roots, inside the endorhiza, but not inside leaves (with or without *B. cinerea* leaf inoculation; Suppl. 1).

The microscopic analyses of FISH experiments (Fig. 1) demonstrated that the strain NRRL B-24137, 10 days post-inoculation, had colonized the rhizoplane (with a hyphae form), especially at the root hair zone (Fig. 1a, b). No similar finding was recorded in case of control plants (inoculated with PBS) or without hybridization with the probes. The bacterial strain was additionally visualized on the root elongation zone (Fig. 1c, d) as well as very few hyphae at root secondary emergence sites (Fig. 1e, f). Inside the endorhiza (Fig. 1g), the strain NRRL B-24137 was found as hyphae between rhizodermal cells or inside rhizodermal cells (Fig. 1h, i). It was also detected as inter- or intracellular in the cortex region (Fig. 1j, k), where a parietal apposition on some plant cell walls was additionally visualized near the strain (Fig. 1k). In root xylem vessels, the bacterium was not visualized in the lumen of the vessels. Microscopic analysis also showed that the strain NRRL B-24137 could not be detected inside leaves (data not shown).

S. algeriensis NRRL B-24137 protects *A. thaliana* against *B. cinerea* infection

After the study of colonization by strain NRRL B-24137 on and inside *Arabidopsis* seedlings in the presence or absence of *B. cinerea*, the actinobacterial strain was evaluated for its potential to protect the plants

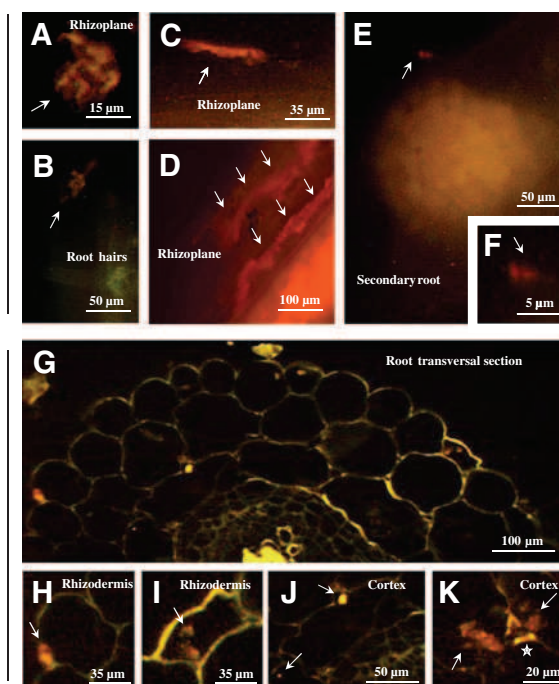
against the necrotrophic agent *B. cinerea* BC1. The results showed that *Arabidopsis* plant roots of wild-type Col (NASC accession number N39005) inoculated with *S. algeriensis* NRRL B-24137 have less surface necrosis per leaf due to *B. cinerea* BC1 than control plants (Fig. 2a, b). A similar finding was obtained when Col-0 (NASC accession number N1092) seedlings were used (Fig. 2b). The lesions were, however, small. All leaves inoculated with the *B. cinerea* isolate did not always show disease symptoms. Leaves for each accession line and treatment showed between 0 mm² (no symptoms) and 6 mm² of surface area infection and scores of no symptoms were included in mean values and standard deviation (also done for the mutants used in this study). The mean surface of necrosis due to *B. cinerea* infection on WT N39005 was 1.12±0.24 mm² of surfaces of necrosis per lesion for control plants, while a surface of 0.33±0.11 mm² per leaf was recorded for seedlings that were root inoculated with NRRL B-24137 (different with *P*<0.05; Fig. 2b). Seedlings previously inoculated with NRRL B-24137 therefore showed an average of 71.5 % protection against *B. cinerea* compared to control plants. For the NASC accession number N1092, surfaces of necrosis per leaf were of 1.4±0.28 mm² for control plants, while 0.78±0.23 mm² was recorded for seedling roots inoculated with NRRL B-24137 (different with *P*<0.05; Fig. 2b), corresponding to an average of 44.29 % protection in comparison to control. Interestingly, more leaves without infection were additionally recorded for seedlings that were root inoculated with NRRL B-24137 in comparison to control treatment (data not shown).

The protection induced by *S. algeriensis* NRRL B-24137 against *B. cinerea* requires EDS4 and EDS8, but not EDS5 and EDS9

In order to know more about the mechanisms that could be involved in the systemic resistance induced by NRRL B-24137, protection of *A. thaliana* seedlings was screened using different plant mutants. Among them, some “enhanced disease resistance” mutants were firstly used. These mutants were *eds4-1* (N3799), *eds8* (N3800), *eds5-1* (N3735), and *eds9-1* (N3736). The results showed that *eds4-1* (with scores of surfaces of necrosis of 1.85±0.26 mm² for NRRL B-24137 versus 1.57±0.25 mm² for control) and *eds8* lines (1.43±0.25 vs. 1.22±0.2 mm²) lost the ability to

Fig. 1 Rhizosphere and endophytic colonization of *A. thaliana* cv. Columbia seedlings (WT Col N39005) by *S. algeriensis* NRRL B-24137, analyzed by FISH microscopy, showing (arrows) different forms of the strain (sporulated, hyphae, pellets of hyphae) on the root surfaces at the root hair zone (a, b), root elongation zone (c, d), root emergence site (e, f), and inside the endorhiza (g, k) of the seedlings

Root surfaces of WT Col N39005



Endorhiza of WT Col N39005

be protected against *B. cinerea* (data not different to control treatment with $P>0.05$). However, with *eds5-1* and *eds9-1* lines, the results showed that the resistance induced by NRRL B-24137 is maintained in these two mutants (data between NRRL B-24137 and control treatment different with $P<0.05$; Fig. 3a).

The state of plant resistance induced by *S. algeriensis* NRRL B-24137 requires EIN2, EIN4, and EIN5

Some *Arabidopsis* mutants involved in ethylene signaling were used to determine if gene products related to ethylene signaling could be required for the resistance, induced by strain NRRL B-24137, and enabling growth reduction of *B. cinerea*. Mutant lines *ein2-1* (N8844) and *ein4* (N8053) showed stronger susceptibility to *B. cinerea* in comparison to WT Col, and mutant line *ein5-1* (N8054) showed a slight increase of susceptibility in comparison to accession N-39005 but not Col-0 (N1092). In the case of inoculation with NRRL B-24137, the results showed that the mutants *ein2-1*, *ein4*, and *ein5-1* completely lost their ability to be protected against *B. cinerea* (results not different between control and NRRL B-24137 treatment, with $P>0.05$), in comparison to wild-type seedlings and mutant plants not inoculated with the beneficial strain (Fig. 3b).

The state of resistance induced by *S. algeriensis* NRRL B-24137 requires JAR1 and AOS

To continue to describe putative mechanisms involved in the state of resistance induced by NRRL B-24137, the mutant lines *jar1-1* (N8072) and *aos* (N6149) were used. In the case of control inoculation, the use of *jar1-1* showed a slight increase of susceptibility to *B. cinerea* in comparison to N39005 WT plants (but surprisingly not in comparison to Col-0) whereas *aos* mutant did not in comparison to its background Col6 (*gl-1*) (accession number N8155). When inoculated with NRRL B-24137, mutant lines *jar1-1* and *aos* completely lost their ability to be protected against *B. cinerea*, in comparison to control plants and background line (Col6 *gl-1*) of the *aos* mutant (data not different between control and NRRL B-24137 treatment with $P>0.05$; Fig. 3c).

The protection induced by *S. algeriensis* NRRL B-24137 is NPR1 dependent

Mutants *npr1* which normally lose resistance in both SAR and ISR phenomena were also used in this study. The results showed that mutants *npr1-1* (N3726) and *npr1-3* (N3802) were not protected against *B. cinerea* BC1 when they were previously inoculated with the

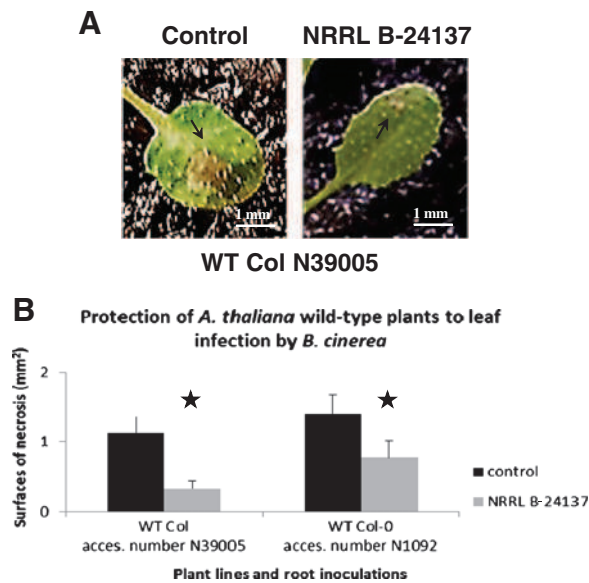


Fig. 2 Protection of *A. thaliana* cv. Columbia wild-type plants against *B. cinerea* by *S. algeriensis* NRRL B-24137. **a** Pictures of *B. cinerea* symptoms on WT Col N39005. **b** Surfaces of leaf necrosis due to *B. cinerea* on WT Col N39005 and WT Col N1092 after root inoculation with NRRL B-24137 or not. * $P < 0.05$ indicates different

strain NRRL B-24137 (results not different between control and NRRL B-24137 treatment with $P > 0.05$; Fig. 3d).

The protection induced by *S. algeriensis* NRRL B-24137 also requires PAD1 but not PAD3 nor PAD4

Experiments were carried out on some phytoalexin mutants to determine if the gene products could be required for the state of resistance induced by the strain NRRL B-24137. The results showed that control inoculation of mutant lines *pad1* (JA-dependent; N3803) and *pad4-1* (SA-dependent; N3806) did not show any difference with WT plants whereas the use of *pad3-1* (SA-dependent; N3805) resulted in a slight increase. Mutant *pad1* completely lost its ability to be protected against *B. cinerea* normally induced by NRRL B-24137 (data not different between control and NRRL B-24137 treatments with $P > 0.05$; Fig. 4). However, *pad3-1* (SA-dependent) and *pad4-1* (SA-dependent) did not completely lose their abilities to be protected against *B. cinerea* (results different between control and NRRL B-24137 treatments with $P < 0.05$; Fig. 4).

UPS1 is required for the ISR induced by *S. algeriensis* NRRL B-24137

The camalexin *ups1* mutant (N6582), defective in a wide range of defense responses due to SA and JA/ET signaling reduction, and also compromised in ROS-mediated gene expression, was additionally used in this study. When the mutant plant was inoculated with strain NRRL B-24137, the protection against *B. cinerea* BC1 was completely abolished in comparison to wild-type plants (data between control and NRRL B-24137 treatment of *ups1-1* not different with $P > 0.05$; Fig. 5a).

AtrbohF is required for the ISR induced by *S. algeriensis* NRRL B-24137 and *AtrbohD* seems to be involved

To continue to describe putative mechanisms involved in the resistance, we evaluated the implication of *AtrbohD* and *AtrbohF* in our ISR model. The results showed that the use of *AtrbohD-3* (N9555) resulted in an increase of susceptibility to *B. cinerea* in comparison to WT plants whereas *AtrbohF-3* (N9557) and surprisingly the double mutant *AtrbohDF-3* (N9558) did not. When these mutants were inoculated with NRRL B-24137, the resistance against *B. cinerea* was completely abolished in comparison to wild-type plants (data between control and NRRL B-24137 treatment not different with $P > 0.05$; Fig. 5b), except for mutant *AtrbohD-3*. When mutant *AtrbohD-3* was used on two sets of plants, the protection was fully abolished (data between control and NRRL B-24137 treatment not different with $P > 0.05$) but not completely in another set (although strongly reduced in comparison to WT plants but with differences with $P < 0.05$). Means of these three sets results in difference with $P < 0.05$ due to this later plant set. However percent of leaves with infection after using this mutant line were similar between control and NRRL B-24137 treatments (data not shown).

Discussion

In this study, the interaction between *A. thaliana* plants and a soil bacterium from a palm grove in Algeria was

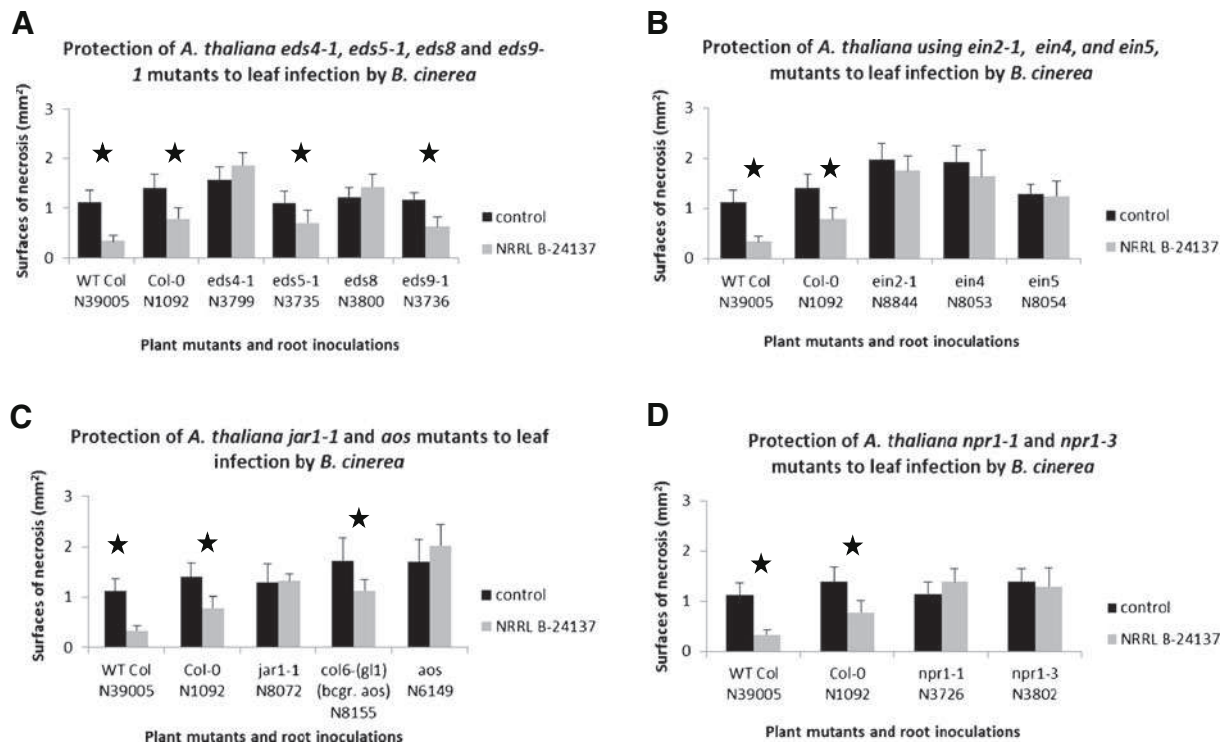


Fig. 3 Analysis of protection of *A. thaliana* cv. Columbia mutant lines **a** *eds4-1*, *eds5-1*, *eds8*, *eds9*; **b** *ein2-1*, *ein4*, *ein5*; **c** *jar1-1*, *aos*; and **d** *npr1-1*, and *npr1-3* to *B. cinerea* following

inoculation of *S. algeriensis* NRRL B-24137. * $P < 0.05$ indicates different

investigated in terms of resistance against the necrotrophic agent *B. cinerea*. We firstly showed that the actinobacterial strain *S. algeriensis* NRRL B-24137 could colonize the root surfaces of *Arabidopsis* seedlings and could establish endophytic subpopulations inside the root system of the seedlings. Several studies have demonstrated the root surface colonization as well as the endophytism of some bacterial strains on and inside host or nonhost plants (Hallmann 2001; Compant et al. 2005; 2010a; Rosenblueth and Martínez-Romero 2006). Some of these bacteria can colonize the systemic plant parts, while others are restricted to the root systems (Compant et al. 2010a; Rosenblueth and Martínez-Romero 2006). In this study, we showed that the strain NRRL B-24137 is restricted to the root level. A systemic colonization later on (e.g., several weeks after root inoculation) cannot, however, be excluded and could be possible. To study mechanisms of resistance induced by beneficial bacteria, it is preferable that the biocontrol strain is absent from the site of infection of the phytopathogen (described by van Loon et al. 1998). As strain NRRL B-24137 can reduce *B.*

cinerea growth directly (Lamari 2006), this colonization process needed to be studied before demonstrating if plant resistance induced by NRRL B-24137 could take place.

It was demonstrated in this study that the strain NRRL B-24137 could confer leaf protection against *B. cinerea*. The infection was low using the strain *B.*

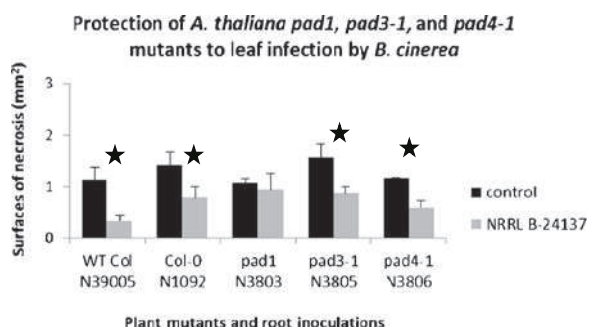


Fig. 4 Analysis of *A. thaliana* cv. Columbia mutant lines *pad1*, *pad3-1*, and *pad4-1* protection to *B. cinerea* following inoculation of *S. algeriensis* NRRL B-24137. * $P < 0.05$ indicates different

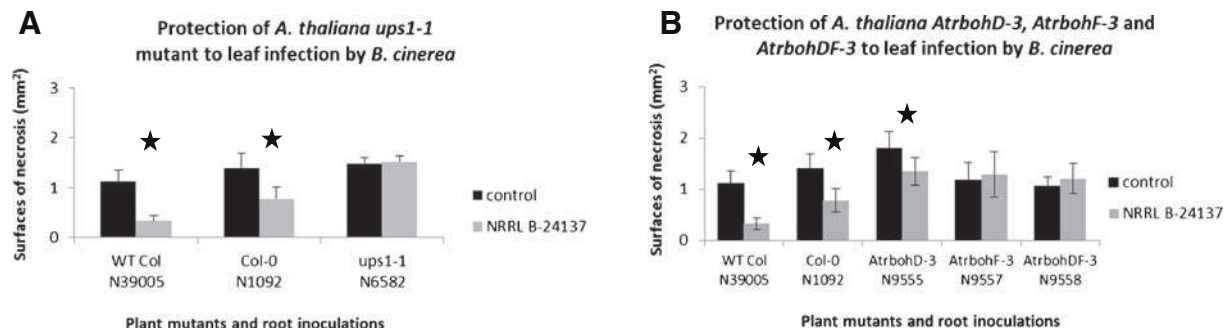


Fig. 5 Analysis of protection to *B. cinerea* following inoculation of *S. algeriensis* NRRL B-24137 on some other *A. thaliana* cv. Columbia mutant lines. Mutants are **a** *ups1-1* and **b** *AtrbohD-3*, *AtrbohF-3*, and double mutant *AtrbohDF-3*. * $P < 0.05$ indicates different

cinerea BC1 and *A. thaliana* seedlings. This can, however, depend to the virulence of the *B. cinerea* strain that was isolated from another plant (grapevine) and not from *Arabidopsis*. Although this virulence was low, this study demonstrated that NRRL B-24137 can confer resistance against *B. cinerea*.

We further evaluated parts of the mechanisms involved in the resistance phenomenon using *Arabidopsis* seedling mutants. This study has evaluated that some genes products related to ET sensitivity and JA signaling are required for the systemic resistance induced by the beneficial endophyte. All the mutants used in this study lose their resistance against phytopathogens once inoculated with a beneficial bacterium (Kloepper et al. 2004; Pieterse et al. 1996, 1998). However, as other beneficial bacteria can trigger only SA pathway mechanisms, this needed to be evaluated with *S. algeriensis* NRRL B-24137. Some rhizobacteria do not induce systemic resistance via the JA/ET pathway but rather via the SA pathway (see for instance Maurhofer et al. 1994, 1998; De Meyer and Höfte 1997; De Meyer et al. 1999; Audenaert et al. 2002; Barriuso et al. 2008; van de Mortel et al. 2012). The SA-dependent signaling pathway results in local and systemic increases in endogenously synthesized SA, leading to activation of the regulatory protein *nonexpressor of pathogenesis-related* (PR) genes1 (*npr1*) and subsequent expression of genes encoding PR proteins, including PR1, PR2, and PR5 (Ward et al. 1991; van Loon and van Strien 1999). Using various mutants in our study, we showed that products of *jar1*, *aos*, *eds8*, and *pad1* that are JA-dependent are required for the resistance induced by NRRL B-24137 against *B. cinerea*. The products of *ein4*, *ein2*, *ein5*, and *eds4* that are ET dependent are also required. We investigated whether the SA pathway could also be associated with the plant resistance

induced by NRRL B-24137 using *Arabidopsis* mutants, and showed that *eds5*, *eds9*, *pad3*, and *pad4*, whose expression are SA dependent, are not involved in the resistance induced by NRRL B-24137. Using the mutant NahG, we additionally showed that the mutant does not lose the ability to be resistant against *B. cinerea* once inoculated with NRRL B-24137 (unpublished results). The resistance was, however, reduced but not completely abolished and it is possible that there are some crosstalks between SA and JA/ET pathways and that they are involved in our model. This mutant is known to have pleiotropic effects and that reduced NahG could lead to other effects (Heck et al. 2003). Other mutants like, for example, *sid2-1* and *sid 2-2* lines that have compromised SA signaling were however not tested and this needs further works.

Using *npr1* mutants, we demonstrated that *npr1-1* and *npr1-3* have their ability to be protected against *B. cinerea* BC1 completely abolished when they have been previously inoculated with the strain NRRL B-24137. Compared with *npr1-1*, which is impaired in JA/ET/SA signaling, *npr1-3* is only impaired in SA signaling (Cao et al. 1994, 1997), confirming the previous hypothesis.

We further evaluated in this study if some gene products not previously reported in ISR could be involved in the resistance observed against *B. cinerea*. In particular, the mutant *ups1* was used. UPS1 has been demonstrated as being involved in resistance induced by abiotic stresses (Ferrari et al. 2007) as well as by phytopathogens (Denby et al. 2005) but never by beneficial microbes. In this study, it is demonstrated that UPS1 integrity is required for the systemic resistance induced by NRRL B-24137, adding therefore to previous models a new component of ISR. *ups1* appears to encode a regulatory protein required for the expression

of different defense genes activated by reactive oxygen species (Denby et al. 2005). As it has been suggested that UPS1 acts downstream of ROS signaling (Denby et al. 2005), we evaluated if the respiratory burst oxidases RBOHD and RBOHF involved in ROS signaling could be involved in the ISR induced by the strain NRRL B-24137. The results showed that RBOHF is required (and possibly RBOHD) for the ISR induced by the strain NRRL B-24137 against *B. cinerea*, adding a new component to our model of ISR induced by NRRL B-24137. It is still unknown if UPS1, RBOHD, and RBOHF are produced during the interaction between the plants and the beneficial strain or if the related genes are primed during *B. cinerea* infection. Their involvements were only characterized through the use of seedling mutants.

Several bacteria have been shown to be inducers of systemic resistance towards phytopathogens and the *Arabidopsis* model has been extensively used (van Loon and Bakker 2004, 2005). For instance, strains of *Pseudomonas* spp. and *Bacillus* spp. (Pieterse et al. 1996; Hammerschmidt 1999; Ryu et al. 2004) as well as many others, including *Actinobacteria* (Conn et al. 2008), have been characterized as inducers of plant resistance. However, none of these studies relate to the use of the *Saccharothrix* genus and systemic resistance mechanisms. This bacterium was isolated from a palm grove with desert soil and the results showing protection against *B. cinerea* suggest that a reservoir of new inducers of resistance could be found in such extreme environments. It is known that microbes from desert soil can help plants tolerate abiotic stresses, as has been demonstrated with pepper plant and drought stress (Marasco et al. 2012). Desert farming also leads to a drastic shift in the bacterial communities in desert soil and indigenous desert microorganisms promote plant health in desert agro-ecosystems (Köberl et al. 2011). On other kinds of ecosystems, effects of the use of bacteria from desert soil have been demonstrated. The beneficial endophytic fungus *Piriformospora indica* isolated from an Indian desert soil can reduce drought stress and other abiotic stresses on several plants, and could also confer plant resistance against phytopathogens (Oelmüller et al. 2009). *Streptomyces* isolated from Algerian Saharan soils could control *Fusarium culmorum* on barley seedlings (Yekkour et al. 2012). Some other studies could be found regarding the use of bacteria isolated from harsh environments and their uses on nonhost plants. Taking into

account the high potential of such microbes to alleviate abiotic and biotic stresses, it could be very interesting however to study more strains of these kinds of microbes and their putative interaction with nonhost plants for plant improvement as well as use *A. thaliana* plants to understand mechanisms involved.

The ability of the soil bacterium *S. algeriensis* NRRL B-24137 to protect plants against *B. cinerea* on *Arabidopsis* seedlings and the mechanisms involved were partly characterized. However, further studies are needed to better understand all the interaction between strain NRRL B-24137 and *A. thaliana*. Additional mutants can be also screened. The strain NRRL B-24137 also secretes a large variety of secondary metabolites having antifungal properties (Lamari et al. 2002a, b; Brandam et al. 2008). It is possible that these metabolites may also act as molecular determinants of ISR. Further research will give more in-depth knowledge on *S. algeriensis* NRRL B-24137 plant resistance mechanisms.

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References

- Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol* 56: 1919–1925
- Audenaert K, Pattery T, Cornelis P, Höfte M (2002) Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. *Mol Plant Microbe Interact* 15:1147–1156
- Bakker PAHM, Pieterse CMJ, Van Loon LC (2007) Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* 97:239–243
- Barriuso J, Solano BR, Gutiérrez Mañero FJ (2008) Protection against pathogen and salt stress by four plant growth-promoting rhizobacteria isolated from *Pinus* sp. on *Arabidopsis thaliana*. *Phytopathology* 98:666–672
- Barrow JR, Lucero ME, Reyes-Vera I, Havstad KM (2008) Do symbiotic microbes have a role in plant evolution, performance and response to stress? *Com Integr Biol* 1:69–73

- Brandam C, Lebrhi A, Meyer XM, Strub C (2008) *Saccharothrix algeriensis*, a new antibiotic producer: investigations on its secondary metabolism. In: Industrial Biotechnology International Conferences, 8–11 June 2008, Italy.
- Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* 6:1583–1592
- Cao H, Glazebrook J, Clarke JD, Volko S, Dong X (1997) The *Arabidopsis* NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* 88:57–63
- Compant S, Clément C, Sessitsch A (2010a) Plant growth-promoting bacteria in the rhizo- and endosphere of plants. Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol Biochem* 42:669–678
- Compant S, Duffy B, Nowak J, Clément C, Ait Barka E (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl Environ Microbiol* 71:4951–4959
- Compant S, Reiter B, Colli-Mull JG, Gangl H, Sessitsch A (2011) Endophytes of grapevine flowers, berries and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb Ecol* 62:188–197
- Compant S, van der Heijden M, Sessitsch A (2010b) Climate change effects on beneficial plant–microbes interactions. *FEMS Microbiol Ecol* 73:197–214
- Conn VM, Walker AR, Franco CMM (2008) Endophytic actinobacteria induce defense pathways in *Arabidopsis thaliana*. *Mol Plant-Microbe Interact* 21:208–218
- Daims H, Brühl A, Amann R, Schleifer K-H, Wagner M (1999) The domain-specific probe EUB338 is insufficient for the detection of all bacteria: development and evaluation of a more comprehensive probe set. *Syst Appl Microbiol* 22:434–444
- De Meyer G, Capieau K, Audenaert K, Buchala A, Métraux JP, Höfte M (1999) Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. *Mol Plant Microbe Interact* 12:450–458
- De Meyer G, Höfte M (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathology* 87:588–593
- Denby KJ, Jason LJM, Murray SL, Last RL (2005) *ups1*, an *Arabidopsis thaliana* camalexin accumulation mutant defective in multiple defence signaling pathways. *Plant J* 41:673–684
- Ferrari S, Galletti R, Denoux C, De Lorenzo G, Ausubel FM, Dewdney J (2007) Resistance to *Botrytis cinerea* induced in *Arabidopsis* by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. *Plant Physiol* 144:367–379
- Glazebrook J, Chen W, Estes B, Chang H-S, Nawrath C, Métraux J-P, Zhu T, Katagiri F (2003) Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. *Plant J* 34:217–228
- Hallmann J (2001) Plant interactions with endophytic bacteria. In: Jeger MJ, Spence NJ (eds) *Biotic interactions in plant–pathogen associations*. CABI, Wallingford, pp 87–119
- Hallmann J, Berg B (2007) Spectrum and population dynamics of bacterial root endophytes. In: Schulz BJE, Boyle CJC, Sieber TN (eds) *Microbial root endophytes*. Springer, Berlin, pp 15–31
- Hammerschmidt R (1999) Induced disease resistance: how do induced plants stop pathogens. *Physiol Mol Plant Pathol* 55:77–84
- Hann J, Kamber M (2000) *Data mining: concepts and techniques*. Morgan Kaufman, San Francisco
- Heck S, Grau T, Buchala AJ, Métraux JP, Nawrath C (2003) Genetic evidence that expression of NahG modifies defence pathways independent of salicylic acid biosynthesis in the *Arabidopsis–Pseudomonas syringae* pv. tomato interaction. *Plant J* 36:342–352
- Kloepper JW, Ryu C-M, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94:1259–1266
- Köberl M, Müller H, Ramadan EM, Berg G (2011) Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. *PLoS ONE* 6(9):e24452
- Lamari L, Zitouni A, Boudjella H, Badjin B, Sabaou N, Lebrhi A, Lefebvre G, Seguin E, Tillequin F (2002a) New dithiopyrrolones antibiotics from *Saccharothrix* sp. SA 233. I. Taxonomy, fermentation, isolation and biological activities. *J Antibiot* 55:696–701
- Lamari L, Zitouni A, Dob T, Sabaou N, Lebrhi A, Germain P, Seguin E, Tillequin F (2002b) New dithiopyrrolone antibiotics from *Saccharothrix* sp. SA 233. II. Physicochemical properties and structure elucidation. *J Antibiot* 55:702–707
- Lamari L (2006) *Production de nouveaux antibiotiques du groupe des pyrrothines par une nouvelle espèce dactinomycète, Saccharothrix algeriensis*. Thèse de doctorat, E.N.S. de Kouba (Algérie), pp 1–177.
- Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* 63:541–556
- Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Beahry UA, Sorlini C, Cherif A, Zocchi G, Daffonchio D (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. *PLOS ONE* 7(10):e48479
- Maurhofer M, Hase C, Meuwly P, Métraux JP, Défago G (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: influence of the *gacA* gene and of pyoverdine production. *Phytopathology* 84:139–146
- Maurhofer M, Reimann C, Schmidli-Sacherer P, Heeb S, Haas D, Défago G (1998) Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. *Phytopathology* 88:678–684
- Mitter B, Brader G, Afzal M, Compant S, Naveed M, Trognitz F, Sessitsch A (2013) Advances in elucidating plant–soil–microbe (bacteria) interactions. *Adv Agron* 121:381–445
- Muzammil S, Compant S, Yu Z, Mathieu F, Lebrhi A (2011) *Saccharothrix algeriensis* NRRL B-24137: a new endophyte with high potential to protect grapevine towards *Botrytis cinerea* in case of high temperature conditions. In: *Oeno 2011 - Actes de colloques du 9e symposium international d'oenologie de Bordeaux*. Dunod (in press)

- Oelmüller R, Sherameti I, Tripathi S, Varma A (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis* 49:1–17
- Pieterse CM, van der Does D, Zamioudis C, Leon-Reyes A, van Wees SC (2012) Hormonal modulation of plant immunity. *Annu Rev Cell Dev Biol* 28:28–33
- Pieterse CMJ, Van Loon LC (1999) Salicylic acid-independent plant defence pathways. *Trends Plant Sci* 4:52–58
- Pieterse CMJ, Van Loon LC (2004) NPR1: the spider in the web of induced resistance signaling pathways. *Curr Opin Plant Biol* 7:456–464
- Pieterse CMJ, Van Wees SCM, Hoffland E, Van Pelt JA, Van Loon LC (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* 8:1225–1237
- Pieterse CMJ, Van Wees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, Van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell* 10:1571–1580
- Pineda A, Zheng SJ, van Loon JJA, Pieterse CM, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends Plant Sci* 15:507–514
- Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. *Curr Opin Plant Biol* 14(4):435–443
- Roller C, Wagner M, Amann R, Ludwig W, Schleifer KH (1994) In situ probing of gram-positive bacteria with high DNA G+C content using 23S rRNA-targeted oligonucleotides. *Microbiology* 140:2849–2858
- Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interaction with hosts. *Mol Plant-Microbe Interact* 19:827–837
- Ryu C-M, Murphy JR, Mysore K, Kloepper JW (2004) Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against cucumber mosaic virus by a salicylic acid and NPR-1 independent and jasmonic acid-dependent pathway. *Plant J* 39:381–392
- Ton J, De Vos M, Robben C, Buchala AJ, Métraux J-P, Van Loon LC, Pieterse CMJ (2002) Characterisation of *Arabidopsis* enhanced disease susceptibility mutants that are affected in systemically induced resistance. *Plant J* 29:11–21
- Torres MA, Dangl J (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Curr Opin Plant Biol* 8:397–403
- van de Mortel JE, de Vos RC, Dekkers E, Pineda A, Guilloid L, Bouwmeester K, van Loon JJ, Dicke M, Raaijmakers JM (2012) Metabolic and transcriptomic changes induced in *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101. *Plant Physiol* 160(4):2173–2188
- Van der Ent S, Van Wees SCM, Pieterse CMJ (2009) Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. *Phytochemistry* 70:1581–1588
- Van Loon LC, Bakker PAHM (2004) Signaling in rhizobacteria-plant interactions. In: De Kroon J, Visser EJW (eds) *Ecological studies*, vol 168, Root ecology. Springer, Berlin, pp 287–330
- Van Loon LC, Bakker PAHM (2005) Induced systemic resistance as a mechanism of disease suppression by rhizobacteria. In: Siddiqui ZA (ed) *PGPR: biocontrol and biofertilization*. Springer, Dordrecht, pp 39–66
- Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. *Annu Rev Phytopathol* 36:453–483
- van Loon LC, Van Strien EA (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol Mol Plant Pathol* 55:85–97
- Van Wees SCM, Van der Ent S, Pieterse CMJ (2008) Plant immune responses triggered by beneficial microbes. *Curr Opin Plant Biol* 11:443–448
- Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Métraux J-P, Ryals JA (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3:1085–1094
- Yekkour A, Sabaou N, Zitouni A, Errakhi R, Mathieu F, Lebrihi A (2012) Characterization and antagonistic properties of *Streptomyces* strains isolated from Saharan soils, and evaluation of their ability to control seedling blight of barley caused by *Fusarium culmorum*. *Lett Appl Microbiol* 55:427–435
- Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. *Mol Plant-Microbe Interact* 25:139–150
- Zhou N, Tootle TL, Tsui F, Klessig DF, Glazebrook J (1998) PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. *Plant Cell* 10:1021–1030
- Zitouni A (1995) Les genres *Nocardioopsis* et *Saccharothrix* (Actinomycetales) dans les sols sahariens: taxonomie numérique, extraction, purification et caractérisation de quelques antibiotiques synthétisés. Magister de microbiologie, E.N.S. de Kouba, Algeria
- Zitouni A, Boudjella H, Lamari L, Badji B, Mathieu F, Lebrihi A, Sabaou N (2005) *Nocardioopsis* and *Saccharothrix* genera in Saharan soils in Algeria: isolation, biological activities and partial characterisation of antibiotics. *Res Microbiol* 156:984–993
- Zitouni A, Lamari L, Boudjella H, Badji B, Sabaou N, Gaouar A, Mathieu F, Lebrihi A, Labeda DP (2004) *Saccharothrix algeriensis* sp. nov., isolated from Saharan soil. *Int J Syst Evol Microbiol* 54:1377–1381