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Induction of systemic resistance in tomato by *N*-acyl-L-homoserine lactone-producing rhizosphere bacteria

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ABSTRACT

_N_-acyl-L-homoserine lactone (AHL) signal molecules are utilized by Gram-negative bacteria to monitor their population density (quorum sensing) and to regulate gene expression in a density-dependent manner. We show that *Serratia liquefaciens* MG1 and *Pseudomonas putida* IsoF colonize tomato roots, produce AHL in the rhizosphere and increase systemic resistance of tomato plants against the fungal leaf pathogen, *Alternaria alternata*. The AHL-negative mutant *S. liquefaciens* MG44 was less effective in reducing symptoms and *A. alternata* growth as compared to the wild type. Salicylic acid (SA) levels were increased in leaves when AHL-producing bacteria colonized the rhizosphere. No effects were observed when isogenic AHL-negative mutant derivatives were used in these experiments. Furthermore, macroarray and Northern blot analysis revealed that AHL molecules systemically induce SA- and ethylene-dependent defence genes (i.e. PR1a, 26 kDa acidic and 30 kDa basic chitinase). Together, these data support the view that AHL molecules play a role in the biocontrol activity of rhizobacteria through the induction of systemic resistance to pathogens.

Key-words: *Alternaria alternata*; *Lycopersicon esculentum* Mill.; *Serratia liquefaciens*; ; quorum sensing; salicylic acid.

INTRODUCTION

Rhizosphere bacteria multiply to high densities on plant root surfaces where root exudates and root cell lysates provide ample nutrients. Sometimes, they exceed 100 times those densities found in the bulk soil (Campbell & Greaves 1990). Certain strains of these plant-associated bacteria stimulate plant growth in multiple ways: (1) they may fix atmospheric nitrogen; (2) reduce toxic compounds; (3) synthesize phytohormones and siderophores; or (4) suppress pathogenic organisms (Bloemberg & Lugtenberg 2001). Research on the 'biocontrol' activity of rhizobacteria has seen considerable progress in recent years. Disease suppression of soilborne pathogens includes competition for nutrients and production of antimicrobial compounds or lytic enzymes for fungal cell walls or nematode structures (Persello-Cartieaux 2003). By contrast, systemic resistance can also be induced by rhizosphere-colonizing *Pseudomonas* and *Bacillus* species where the inducing bacteria and the challenging pathogen remained spatially separated excluding direct interactions (Van Loon, Bakker & Pieterse *et al*1998; Ryu *et al*. 2004).

A well-characterized system of rhizobacteria-induced resistance between *Arabidopsis thaliana* and *Pseudomonas fluorescens* strain WCS417 has been elaborated by Van Loon's group (Pieterse *et al*. 1996; Van Loon *et al*. 1998). This type of resistance was termed 'induced systemic resistance' (ISR) in order to differentiate it from the pathogen-induced and salicylate-mediated 'systemic acquired resistance' (SAR) (Van Loon *et al*. 1998; Shirasu & Schulze-Lefert 2000). During ISR, colonization with rhizobacteria leads to an enhanced expression ('priming') of jasmonate-inducible genes (Van Wees *et al*. 1999). With the help of various biosynthesis and perception mutants for plant signal molecules, it was possible to demonstrate that ISR requires functional jasmonate and ethylene signalling (Van Loon *et al*. 1998; Pieterse *et al*. 2001).

Bacteria have developed efficient systems to perceive their environment by means of diffusible signals. Well-characterized molecules are *N*-acyl-L-homoserine lactones (AHLs), which occur in various Gram-negative bacteria. They are used as signal molecules to control expression of various functions in a cell density-dependent manner, a phenomenon known as 'quorum sensing'. The structures of AHL molecules discovered to date vary in size and compo-

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sition of their acyl chains. Molecules with chain lengths of 4–16 carbon atoms, which often contain an oxo or hydroxyl group on the third carbon, have been described (Eberl 1999). The strain mainly used in this study, *Serratia liquefaciens* MG1, produces two AHL molecules [*N*-butanoyl (BHL) and *N*-hexanoyl homoserine lactones (HHL)] with the aid of the AHL synthase SwrI (Eberl *et al*. 1996). Swarming motility, a special form of bacterial surface locomotion, the production of extracellular proteolytic and chitinolytic activity as well as the ability to form biofilms on abiotic surfaces, has been shown to be regulated by quorum sensing in this strain (Eberl, Molin & Givskov 1999; Riedel *et al*. 2001; Labbate *et al*. 2004).

AHL-based quorum sensing systems have been identified in many plant-associated bacteria. These systems are involved in the regulation of Ti plasmid transfer in *Agrobacterium tumefaciens*, the production of wall-degrading enzymes in the soft-rot phytopathogen *Erwinia carotovora*, as well as the production of virulence factors, antibiotics and biofilm formation (Pierson III, Wood & Pierson 1998; Eberl 1999).

In recent years, evidence has accumulated that AHL molecules are also perceived by eukaryotes, which in turn often specifically respond to these bacterial signals. AHLs were found to stimulate inflammatory and immunogenic responses in mice or to affect IL-8 production in human cells, suggesting that AHLs may act as virulence factors (Smith *et al*. 2001). Mathesius *et al*. (2003) showed that *Medicago truncatula* roots respond to AHL exposure by significant changes in the accumulation of over 150 proteins, depending on AHL structure, concentration and time of exposure. AHL-mimicking compounds and AHL inhibitory activities were reported in exudates from pea seedlings (Teplitski, Robinson & Bauer 2000; Gao *et al*. 2003).

The aim of this study was to investigate whether AHL can contribute to the biocontrol activity of typical rhizobacteria. For this purpose, AHL-producing and AHL-deficient strains were compared in terms of their effects on systemic resistance of tomato to the fungal leaf pathogen *Alternaria alternata*. AHL-producing rhizobacteria as well as AHL molecules were then examined for interference with plant signaling pathways. In addition, expression of plant defence genes was analysed after treatment with AHL molecules. The results suggest that tomato plants are able to detect the presence of typical rhizobacteria using AHL molecules, and to respond by systemic induction of defence genes.

MATERIALS AND METHODS

Plant material and growth conditions

Tomato plants (*Lycopersicon esculentum* Mill. cv. Micro-Tom, Bruno Nebelung GmbH, Everswinkel, Germany) were either grown in potting substrate or in 250 g autoclaved quartz sand inside sterile plastic containers (PhytaTray, Sigma, Taufkirchen, Germany). Fifteen PhytaTrays containing five plants each were placed in a tray covered with a transparent lid to reduce contamination. The plants were watered with nutrient solution according to Steidle *et al*. (2001). Prior to sowing, the seeds were surface-sterilized with 5% (w/v) sodium hypochlorite for 20 min. The plants were incubated under 14 h light [from 0600 h to 2000 h, photosynthetic photon flux density (PPFD) 120 mol m^{-2} s^{-1}, 25 ± 1 °C]/10 h dark (20 ± 1 °C) cycles and 70 ± 5% relative humidity (Tuomainen *et al*. 1997). Middle-aged leaves of 4- (quartz sand culture) to 6-week-old plants (potting substrate) were harvested, immediately frozen in liquid nitrogen and stored at –75 °C.

Inoculation with bacteria and challenge with pathogenic fungi

Green fluorescence protein (GFP)-tagged *S. liquefaciens* strains MG1 and MG44 (Eberl *et al*. 1996) as well as *Pseudomonas putida* strains IsoF (Gfp) and F117 (DsRed) (Steidle *et al*. 2002) were grown overnight at 37 ° and 30 °C, respectively, in Luria-Bertani (LB) medium. After centrifugation at 6000 *g*, the bacteria were resuspended in 1/10 volume 10 mM $MgSO_4$. Ten milliliters of this suspension ([10^{10} cfu mL^{-1})] was added to 5-week-old non-sterile plants by pipetting the bacteria around the shoot onto the soil substrate. Contamination of leaves and shoots was carefully avoided. To determine the colony forming units present in tomato roots, the potting substrate was removed using 1 × phosphate-buffered saline (PBS). Serial dilutions were plated on LB agar supplemented with 50 mg kanamycin L^{-1}, using kanamycin resistance as marker. Cell numbers were determined by counting green fluorescing cells of *gfp*-labelled *S. liquefaciens* compared to the total cell numbers after 4′-6-diamidino-2-phenylindole (DAPI) staining. For this purpose, rhizosphere root samples were homogenized in 4% (w/v) sucrose solution, and 5–10 μL of this suspension was immobilized on polycarbonate filters (GTTP 0.2 μm, Millipore, Eschborn, Germany). The filters were stained in 7×10^{-5}% DAPI for 10 min, gently washed in distilled water and then air-dried. Cell counting was conducted using an epifluorescence microscope (Axioplan2, Zeiss, Oberkochen, Germany).

Three days after the addition of bacteria, the plants were challenged with the leaf pathogenic fungus *A. alternata*. Fungal spores were suspended in 5.5 mM $KHPO_4$, 62.5 mM glucose, 0.1% (v/v) Tween 20 (pH 6.0; modified after Leone & Tonneijk 1990) and sprayed onto the leaves (1000 spores mL^{-1}). Leaf injury was determined independently by two persons using visual estimation of percentage necrotic leaf area.

Confocal laser scanning microscopy

Bacterial localization on the rhizoplane was studied using bacteria marked with GFP (green) or DsRed (red) fluorescent proteins. The chromosomal tagging of *S. liquefaciens* MG1 with GFP and *P. putida* IsoF with DsRed was described previously (Steidle *et al*. 2001). The GFP-based AHL monitor strain *P. putida* F117:Tn5-LAS was generated by random insertion of a transposon cassette based on

components of the *las* quorum sensing system of *Pseudomonas aeruginosa* fused with the *gfp* gene (Steidle *et al*. 2001). Freshly harvested roots were washed with 1 × PBS and placed on a glass slide. After embedding in Citifluor (Citifluor Ltd, Canterbury, UK), the fluorescence was detected using a confocal laser scanning microscope (LSM510, Zeiss) (Hartmann *et al*. 1998).

Treatment with AHL and salicylic acid (SA)

Four-week-old plants grown in sterile quartz sand were treated with 1–10 μM *N*-BHL or HHL (Fluka, Seelze, Germany). A 100 × stock solution in methanol was diluted in 5 mL sterile water and pipetted on the sand to avoid contact with shoots and leaves. Control plants received equal amounts of methanol in water. Treatment with 0.5 mM SA (dilution of 100 × methanol stock in water) was conducted in the same way.

SA determination

Extraction and quantification of free and conjugated SA were performed according to Meuwly & Métraux (1993). SA was detected using a Shimadzu RF 535 fluorescence detector (Shimadzu Europe, Duisburg, Germany) at excitation and emission wavelengths of 305 and 407 nm, respectively. The leaves from plants grown in non-sterile soil as well as shoots and roots from axenically grown plants were analysed.

Isolation of genomic DNA and quantification of fungal DNA in infected leaves

Fungal DNA of infected leaves was extracted from 100 mg homogenized plant material according to Bahnweg *et al*. (1998). The final DNA pellet was dissolved in 100 μL Tris-EDTA (TE buffer). A 1 μL part of this DNA solution was used for quantitative PCR (QPCR, real-time PCR), which was performed in 50 μL reactions with Absolute QPCR SYBR Green® ROX Mix (ABgene, Hamburg, Germany). *A. alternata*-specific primers (10 μM of each primer), A. alt.-F3 (5′ to 3′ TCTAGCTTTGCTGGAGACTC) and A. alt.-R1.1 (5′ to 3′ AGACCTTTGCTGATAGAGAGT), derived from base-sequences of the internal transcribed spacers (ITS) of ribosomal RNA (rRNA) genes were used to generate short amplicons of 95 bp. The primers were specific for *A. alternata* and did not yield amplicons in PCR with DNA from 90 fungal strains, including all major tomato pathogens. The QPCR was performed with the ABI-PRISM 7700 Sequence Detection System, version 1.6 (Applera, Weiterstadt, Germany). Thermocycler conditions were 50 °C/2 min (elimination of uracil DNA), 95 °C/10 min (activation of enzyme), 40 cycles of 95 °C/15 s–60 °C/1 min (denaturation, annealing and amplification). A standard curve was generated for each run with a dilution series of purified and calibrated *A. alternata* genomic DNA (200 ng/20 ng/2 ng/200 pg/20 pg/2 pg/200 fg prepared in a 10 ng μL^{-1} DNA solution, the latter stabilizing highly diluted genomic DNA). The newly generated double-stranded DNA amplicon was quantified measuring the SYBR Green fluorescence intensity after each 60 °C step of the cycler program. Analysis of experiments [i.e. determination of Ct (cycle threshold) values to quantify initial amounts of target DNA] was conducted using the ABI-PRISM 7700 (Böhm *et al*. 1999).

Isolation of total RNA and Northern hybridization

For RNA isolation and Northern hybridization, leaf material from axenically grown plants was used after treatment with AHL molecules. Total plant RNA was isolated from 100 to 120 mg leaf material using the TRIZOL method according to the manufacturer's manual (Invitrogen, Karlsruhe, Germany). For Northern blot experiments, 5 μg total RNA was separated on a 1% (v/v) formaldehyde gel and transferred onto nylon membranes (Roche, Mannheim, Germany) by capillary blot (Sambrook & Russel 2001). The probes directed against tomato defence genes were cloned into the standard vector pGEM-T (Promega, Madison, WI, USA). The labelling was carried out in PCR reactions using the PCR Dig probe synthesis kit (Roche). Hybridization was performed overnight at 50 °C.

Macroarray analysis

The array used in this study was developed in the course of the European Union (EU)-funded project TOMSTRESS, and will be described elsewhere. The 70 spotted PCR products were directed against tomato defence genes, genes of ethylene biosynthesis and perception, as well as genes involved in developmental processes such as ripening. Each probe was spotted in triplicate. Before using the filters in complex hybridizations, reference hybridization was performed to check the quality of the filters (Thimm *et al*. 2001). For this purpose, a ^{33}P-labelled PCR product-specific primer (T7 oligonucleotide) was hybridized overnight at 38 °C. To remove radioactivity, the membranes were washed twice in 0.4 M NaOH for 30 min at room temperature and then neutralized in 2 × SSC.

The preparation of ^{33}P-labeled complementary DNA (cDNA) using 4 μg total RNA was carried out with the Strip-EZ™ RT Kit (Ambion, Austin, TX, USA). Filters were pre-hybridized for at least 4 h at 65 °C in 5 × SSC, 5 × Denhardt solution, 0.5% (w/v) SDS and 100 μg mL^{-1} salmon sperm DNA. Hybridization of ^{33}P-labelled cDNA was performed under the same conditions overnight. Arrays were then successively washed for 20 min at 65 °C in 20 × SSC, 0.1% SDS and 0.2 × SSC, 0.1% SDS. Radioactive signals were detected using a phosphoimager (FLA-3000, Fujifilm Europe, Dusseldorf, Germany). Images were directly imported into the ArrayVision software (Imaging Research Inc., St. Catharines, Canada). Normalization of the filters was achieved by dividing the signal intensity of each spot by the signal intensity of the mean of signal intensities of the whole filter. Each gene was spotted in triplicate, and the average of the three values was calcu-

lated. Then, the value for the treated sample was divided by the control value to obtain the induction factors. Only those signal intensities were considered, which were at least twice the background for treated sample or control. The methodical variability of macroarray experiments was determined by hybridizing three independent RNA samples extracted from the same plant material and ranged from 1 to 9% SE. The biological variability was investigated using three independent plant samples and was between 4 and 27%.

Statistical analyses

All experiments were performed at least twice with three replicate samples per test condition. The data shown are from representative experiments. When indicated, Tukey's multiple range test was used to determine differences among the treatment means (at $P < 0.05$; Statgraphics software, STSC, Inc., Rockville, MD, USA).

RESULTS

Systemic resistance to *A. alternata* is induced in tomato by AHL-producing rhizobacteria

When tomato plants (cv. Micro-Tom) were inoculated with the AHL-producing rhizobacterium *S. liquefaciens* MG1, the appearance of necrotic cell death after leaf infection with *A. alternata* was reduced by more than 70% 4 d after pathogen challenge (Figs 1a & 2a). In order to analyse the impact of AHL molecules in this interaction, an isogenic AHL-negative strain (MG44) was used in which the sole AHL synthase gene swrI was mutated by a gene replacement procedure (Eberl *et al*. 1996). Growth of the wild-type MG1 and the AHL-negative mutant MG44 in the rhizosphere was comparable during 9 d post-inoculation (Fig. 3). Inoculation with MG44 slowed down the development of *A. alternata* – induced cell death, but showed no significant alterations in response to the fungal pathogen when compared with non-inoculated controls after 4 d (Figs 1a & 2a).

Previously, we developed GFP-based monitor strains that allow *in situ* visualization of AHL-mediated communication among individual cells in the plant rhizosphere (Steidle *et al*. 2001). Based on constitutive GFP production under the control of an LacI-repressible promotor (Andersen *et al*. 1998), it could be shown that *S. liquefaciens* MG1 colonized the tomato rhizosphere in gnotobiotic systems (Fig. 1b) and in standard substrate (not shown). The bacteria were distributed uniformly over the root surface. The colonization behaviour of the AHL-negative mutant MG44 was similar (not shown).

AHL-mediated cell-to-–cell communication was demonstrated *in situ* between a GFP-based AHL monitor strain (*P. putida* F117:Tn*5*-LAS) and an AHL-producing bacterial isolate from tomato roots (*P. putida* IsoF) tagged with the red fluorescent protein DsRed (Steidle *et al*. 2002). *P. putida* F117 is the AHL-negative derivate of IsoF. Given that the sensitivity of the monitor strains is within 0.1–1 μM,

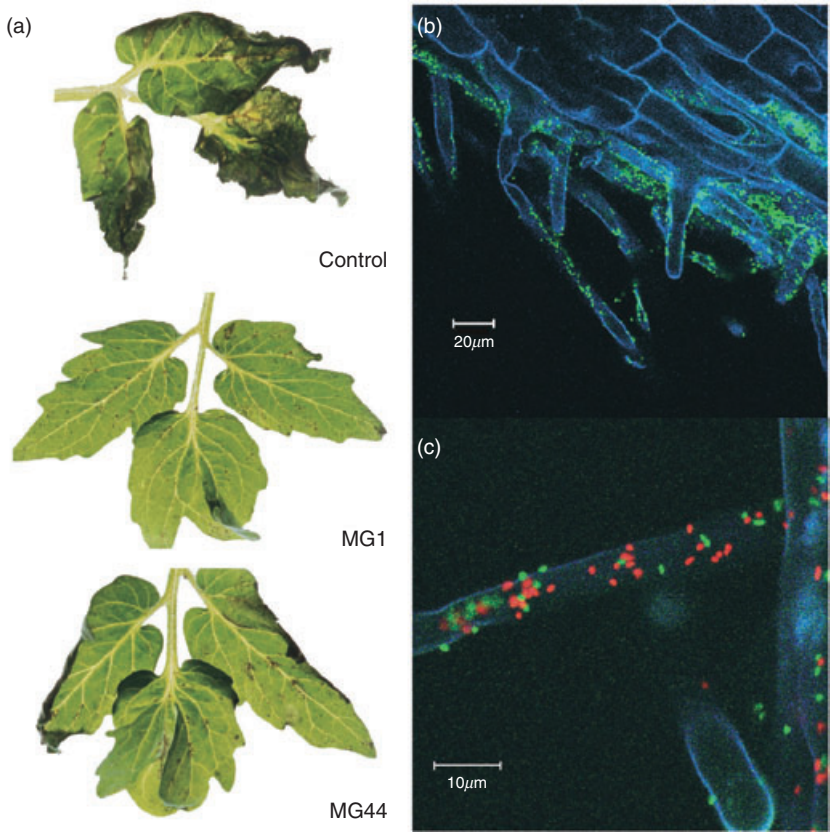

Figure 1. Effects of root inoculation with rhizobacteria on pathogen resistance of tomato plants. (a) Primary leaves of tomato plants grown in potting soil non-treated (control) or pre-inoculated with *Serratia liquefaciens* MG1 (wild type) and MG44 [(*N*-acyl-L-homoserine lactone (AHL)-negative]). Three days after addition of bacteria plants were challenged with *Alternaria alternata*. Pictures were taken 3 days after pathogen treatment. (b) Colonization of tomato roots with green fluorescence protein (GFP)-tagged *S. liquefaciens* MG1. (c) AHL-mediated cell-cell communication in the tomato rhizosphere between a GFP-based AHL monitor strain (green cells, *Pseudomonas putida* F117:Tn*5*-LAS) and an AHL-producing tomato isolate (red cells, *P. putida* IsoF tagged with the red fluorescent protein DsRed). Root samples of axenically grown plants were inspected by confocal laser scanning microscopy (630×).

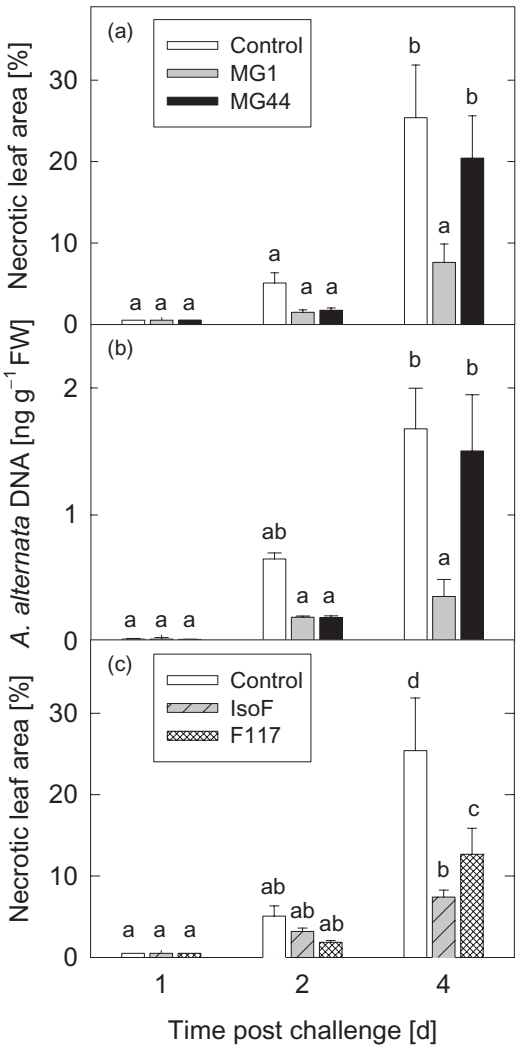

Figure 2. Effects of root inoculation with rhizobacteria on leaf cell death and pathogen growth. Visual estimation of necrotic leaf area (a, c) and quantification of *Alternaria alternata* DNA by Real-Time PCR (b). Five-week- old plants grown in potting soil were inoculated with different strains of rhizobacteria and then challenged with *A. alternata* 3 days later. Bars represent ± S.E., $n = 6$ (a) and 3 (b, c). Means with the same letter are not significantly ($P < 0.05$) different according to Tukey's multiple range test.

AHLs are produced by the microbial community colonizing the tomato rhizosphere in biologically relevant amounts, and the AHL concentrations in the tomato rhizosphere are in the μM–mM range (Fig. 1c). More importantly, no signals were detected when the sensor strains were inoculated alone, excluding the possibility that a plant derived a signal activating the AHL monitor strain (not shown). No GFP-labelled or kanamycin-resistant rhizobacteria (neither *S. liquefaciens* nor *P. putida*) could be detected in the shoots and leaves of the tomato cv. Micro-Tom, suggesting that endophytic growth did not occur.

To determine whether growth of *A. alternata* in tomato leaves was reduced by pre-inoculation of roots with rhizobacteria, we quantified *A. alternata* DNA by real-time PCR. To this end, primers for *A. alternata*, which were based on ITS regions of ribosomal DNA genes, were developed. Plants pre-treated with *S. liquefaciens* MG1 exhibited 79% lower amounts of *A. alternata* DNA when compared with non-inoculated controls (Fig. 2b). In contrast, 4 d after infection, the amount of *A. alternata* DNA was comparable in controls and in plants that were inoculated with the AHL minus mutant MG44. Leaf injury data were positively correlated ($r = 0.85$) with the leaf-–associated amounts of *A. alternata* DNA.

Inoculation with the AHL-producing *P. putida* strain IsoF isolated from the tomato rhizosphere (Steidle *et al*. 2001) also resulted in a marked reduction of leaf damage following challenge with *A. alternata*. This strain produces at least four different 3-oxo-AHL molecules with acyl side chains varying from C6 to C12 with the aid of a single AHL synthase (Steidle *et al*. 2002). There was no significant difference in root colonization between the wild-type strain IsoF ($3.5 \pm 0.8 \times 10^5$ cfu g^{-1} fresh weight (FG)) and the AHL-negative mutant F117 ($5.0 \pm 2.5 \times 10^5$ cfu g^{-1} FG) after 7 d. The quorum sensing system of IsoF is suggested to be involved in the regulation of biofilm development as the AHL-negative mutant F117 formed a structurally aberrant biofilm relative to the one formed by the wild type. In contrast to *S. liquefaciens*, however, the isogenic AHL minus *P. putida* mutant F117 still reduced necrotic cell death to about 50% (Fig. 2c), suggesting that additional factors which are not regulated by quorum sensing compounds contribute to the biocontrol activity of *P. putida* IsoF.

AHL induction of SA production and systemic expression of defence-related proteins

To investigate whether root inoculation with AHL-producing rhizobacteria induces systemic plant responses, we mea-

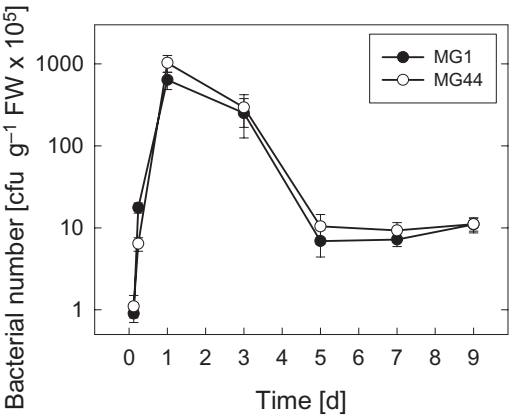

Figure 3. Growth of the *Serratia liquefaciens* wild- type MG1 and the *N*-acyl-L-homoserine lactone (AHL)- negative mutant MG44 on tomato roots. Five-week-old tomato plants grown in potting substrate were root-inoculated with the bacteria on day 0, and bacterial numbers (colony forming units, (cfu)) were counted at the intervals given in the Figure. Bacteria were chromosomally labelled with green fluorescence protein (gfp), which was constitutively expressed and and enabled the detection with fluorescence microscopy. Bars represent ± S.E., $n = 3$.

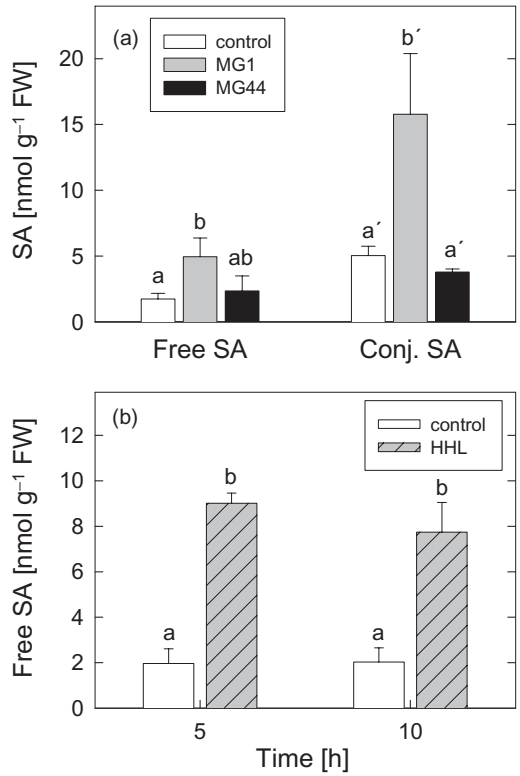

Figure 4. Effects of root inoculation with the rhizobacterium *S. liquefaciens* (a) and of *N*-acyl-L-homoserine lactone (AHL) application (b) on contents of salicylic acid (SA) of tomato plants. (a) Leaf samples for SA acid determination were harvested 3 days after inoculation of soil-grown plants with wild-type MG1 and the AHL-negative mutant MG44 or with 10 mM $MgSO_4$ treatment (control). (b) Contents of free SA in roots after addition of 10 μM *N*-hexanoyl homoserine lactone (HHL). These plants were grown in quartz sand. Bars represent ± S.E., $n = 3$. Means with the same letter are not significantly ($P < 0.05$) different according to Tukey's multiple range test.

sured the levels of the signal molecule SA as well as gene expression in middle-aged tomato leaves. Free, as well as conjugated, SA levels were elevated up to threefold within 3 d after inoculation with *S. liquefaciens* MG1 (Fig. 4a). In addition, levels of conjugated gentisic acid (2,5-dihydroxy benzoic acid), a signal molecule leading to a different pattern of pathogenesis-related (PR) proteins in tomato (Bellés *et al*. 1999), increased in the leaves following rhizobacteria inoculation by 50% (data not shown). The AHL-negative strain MG44 did not induce a significant increase in systemic SA levels in free or conjugated form (Fig. 4a). Root application of *N*-HHL, the major AHL species produced by *S. liquefaciens* MG1 (Eberl *et al*. 1996), at concentrations of 10 μM also led to sustained accumulation (three- to fourfold) of SA in tomato roots (Fig. 4b) and less-efficient accumulation in leaves (data not shown). HHL concentrations of at least 1 μM were necessary to affect SA levels in tomato (data not shown).

To further assess plant defence responses to the inoculation with AHL-producing rhizobacteria, we used a macroarray approach with 70 defence and signalling genes. The gene probes spotted on the array were directed against PR proteins and defence-associated genes, genes involved in jasmonate or ethylene biosynthesis and perception as well as signalling. In addition, genes involved in developmental and metabolic processes were analysed (see additional material).

As summarized in Table 1, root application of *N*-BHL and *N*-HHL for 5 h led to an enhanced systemic expression of specific genes involved in defence, the antioxidant system, ethylene signalling as well as in other functions in the leaves. Inoculati on with both AHL molecules induced marked expression of PR1a (P4), PR1 (P6), as well as 26 kDa acidic chitinase with induction factors between 4 and 18 (Table 1). A putative ascorbate peroxidase and the proteinase inhibitor CEVI57 were also induced by both molecules. Interestingly, the spectrum of responding defence genes was broader in response to HHL than to BHL. This included, in particular, a 30 kDa basic chitinase, which is ethylene responsive (Van Kan *et al*. 1995), P69, a subtilisin-like endoproteinase, the ethylene receptor Le-ETR1 as well as InvLp11, a vacuolar invertase.

The systemic expression of PR proteins in the leaves 5 h after applying BHL and HHL to the roots was confirmed by Northern blot hybridization, and was compared to SA induction in terms of its kinetics. PR1a (P4) gene expression peaked after 5 h of BHL or HHL treatment (10 μM each), which was markedly faster when compared with SA induction (at 0.5 mM; Fig. 5). In line with this result, the expression of the 30 kDa chitinase was also induced after 5–10 h by BHL and HHL, while the response to SA was markedly lower (induced expression after 24 h). Control plants showed only minor expression of both PR1a (between 5 and 24 h) and the 30 kDa chitinase (after 5 h). The induction of the 30 kDa chitinase in control plants after 10–24 h is unresolved and may be a result of the elevated humidity in the aseptic plant system used.

DISCUSSION

AHL compounds in the tomato rhizosphere lead to systemic resistance against *A. alternata*

Using GFP-based monitor strains for *in situ* visualization of AHL-mediated communication among individual cells (Steidle *et al*. 2001), we could demonstrate that the ubiquitous rhizobacterium *P. putida* produces AHL molecules in the tomato rhizosphere. The coinoculation experiments and the application of pure AHL compounds were performed on roots of gnotobiotically grown plants, which excludes contamination with other AHL-producing or metabolizing bacteria. As AHL signal molecules are produced and recognized by numerous microorganisms (Pierson III *et al*. 1998), cross-communication between bacterial populations in complex communities can occur. In addition, root-associated bacteria produce AHL molecules more often than soil-borne bacteria (Pierson III *et al*. 1998; Elasri *et al*. 2001; Steidle *et al*. 2001).

Table 1. Macroarray analysis of defence genes in tomato leaves after application of 10 μm N-hexanoyl (HHL) and N-butanoyl (BHL) homoserine lactones to roots of plants growns in quartz sand. Leaves were harvested after 5 hours.

Gene	Accession	Function	Induction relative maximum	
			HHL	BHL
Defence				
PR1a (P4)	AJ011520.1	Unknown	10	10
PR1 (P6)	M69248	Unknown	6	4
Qa	X74905.1	Acidic glucanase	n. d.	8
Chi3	Z15141	26 kDa acidic chitinase	14	18
Chi9	Z15140	30 kDa basic chitinase	12	2
CEVI57	X94946	Proteinase inhibitor	4	3
P69	X95270	Subtilisin-like endoproteinase	3	1
Antioxidative system				
APx20	Pablo Vera, unpublished	Putative ascorbate oxidase	3	2
Ethylene signalling				
LE-ETR1	U38666	Ethylene receptor	3	1
Others				
SENU3	Z48736	Cysteine proteinase	3	2
Actin	ATU42007		3	n. d.
InvLp11	Z12026	Vacuolar invertase	8	1

PR, pathogenesis-related; HHL, hexanoyl homoserine lactone; BHL, butanoyl homoserine lactone; nd, not detectable.

Laser scanning microscopy of red fluorescent 'AHL producer' and green fluorescent 'AHL sensor' strains revealed that the bacteria conducted AHL-dependent cell-to-cell communication while colonizing the rhizoplane (Fig. 1c). Based on the sensitivity of the sensor strain, it was estimated that the indigenous rhizosphere community produces AHL in overall concentrations ranging from 0.1 to 10 μM, while local hot spots (i.e. microcolonies) might be present with much higher AHL concentrations. No signals were detected when the sensor strains were inoculated alone in aseptically grown tomato, eliminating the possibility that plant-derived signals activated the AHL monitor strain.

Systemic pathogen resistance was analysed with *A. alternata,* a member of the genus *Alternaria*, which includes widespread necrotrophic pathogens causing rot diseases on leaves and fruits of a variety of important agronomic host plants including tomato, potato, cauliflower and citrus fruits (Thomma 2003). The analysis showed a clear dependence on the production of AHL molecules by the rhizobacterium *S. liquefaciens* MG1. Tomato plants inoculated with the isogenic AHL-negative mutant MG44 were not significantly different from control plants with regard to susceptibility (Figs 1a & 2a). The contribution of AHL molecules to enhance plant defence responses was confirmed with another AHL-producing strain, *P. putida* IsoF, an isolate from tomato roots (Steidle *et al.* 2001), which also induced resistance against *A. alternata*. However, the difference between wild-type and AHL-negative strain was not as clear-cut as in the case of *S. liquefaciens* although there was no difference in root colonization. Probably, other bacterial determinants such as specific lipopolysaccharides or flagellin structures (Duijff, Gianinazzi-Pearson & Lemanceau 1997; Felix *et al.* 1999) were effective in eliciting biocontrol activity in addition to the AHL production by *P. putida* IsoF.

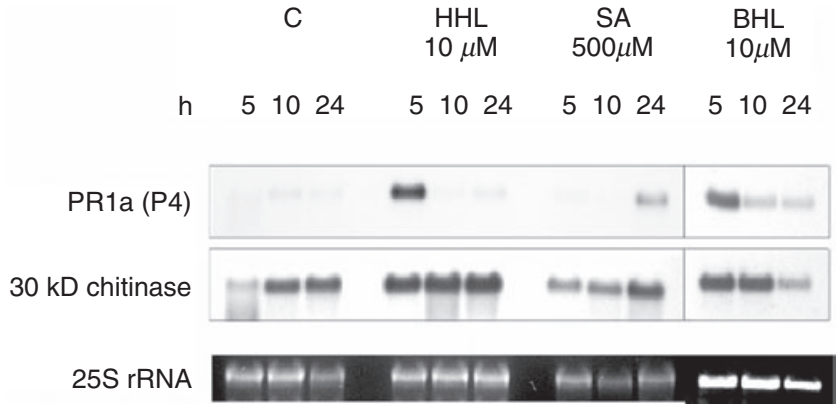

Figure 5. Induction of systemic pathogenesis-related (PR) gene expression in tomato leaves by root application of *N*-hexanoyl (HHL) and butanoyl (BHL) homoserine lactones. *N*-acyl-L-homoserine lactone compounds were applied to the roots of tomato plants grown in quartz sand at 10 μM concentrations while salicylic acid (SA) was applied at 0.5 mM. Northern blots were probed with complementary DNAs for PR1a (P4) and 30 kDa chitinase. Ethidium bromide staining of 25S ribosomal RNA (rRNA) was performed to show equal loading. C, control.

Both visible damage and fungal DNA amounts of *A. alternata* were reduced after pre-inoculation with *S. liquefaciens* MG1 (Fig. 2). Because direct antagonism between rhizobacteria and fungal pathogens is well described (Berg *et al*. 2002), it was important to ensure that biocontrol active bacteria and the pathogen remained spatially separated in our studies. Because GFP-labelled and kanamycin-resistant derivatives of *S. liquefaciens* MG1 or *P. putida* IsoF could not be detected in leaves and shoots, we propose that the plant defence machinery is activated by bacterial components or metabolites in the roots and by subsequent signal translocation to the above-ground parts of the plant.

The role of SA in AHL-induced resistance

Resistance in plants is a highly regulated phenomenon depending on several signalling pathways, each activated by a set of different biotic and abiotic stimuli (Pieterse & van Loon 1999). SA, one of the best characterized signal molecules, seems to be involved in systemic resistance against *A. alternata* induced by *S. liquefaciens* MG1 because elevated levels were systemically measured in leaves after inoculation with the bacteria. In addition, conjugates of gentisic acid were induced by rhizobacteria inoculation as well. Gentisic acid is also pathogen-inducible, and stimulates a pattern of PR proteins in tomato, which is different to that induced by SA (Bellés *et al*. 1999), thereby putatively complementing the response.

Many rhizobacteria of the genus *Pseudomonas* or *Serratia* produce SA under iron-limiting conditions as a precursor of siderophores (De Meyer & Höfte 1997; Press *et al*. 1997; Mercado-Blanco *et al*. 2001). Resistance of tomato or tobacco against *Botrytis cinerea* induced by the biocontrol strain *P. aeruginosa* 7NSK2 depends on *in planta* accumulation of SA as revealed by experiments with transgenic nahG plants, which are not able to accumulate SA (De Meyer, Audenaert & Höfte 1999; Audenaert *et al*. 2002). *S. liquefaciens* MG1 produces picomolar amounts of SA in liquid culture (data not shown), and these low amounts may potentially contribute to induce systemic resistance in plants. However, as the AHL-deficient mutant MG44 failed to effectively induce systemic resistance (Figs 1a & 2a) and did not result in increasing SA levels in leaves (Fig. 4a), a major contribution of SA of bacterial origin can be ruled out in our system. In addition, AHL application to tomato roots led to an induction of SA in roots and, to a lower extent, in leaves (Fig. 4b, data not shown). Therefore, we suggest that AHL-induced SA production is an additional new route to systemic signal transduction in tomato. The possibility that AHL can activate the SAR pathway by damaging cells and tissues can be excluded, as tomato cell suspension cultures were not affected in viability by AHL molecules at the concentrations used. Furthermore, roots and shoots of AHL-treated plants showed no signs of tissue damage. Further experiments with NahG tomato plants should reveal whether MG1 is still able to induce resistance.

Alterations in gene expression induced by AHL molecules

To test whether AHL signal molecules could lead to systemic changes in gene expression, tomato leaves were analysed by macroarray and Northern blot analysis. As shown in Table 1, the expression of several PR proteins was altered by BHL and HHL, two short chain AHL molecules produced by *S. liquefaciens* MG1. The spectrum of genes induced by HHL was broader than that induced by BHL. Both AHL molecules induced isoforms of PR1. In tobacco, acidic and basic isoforms are differentially regulated during defence responses (Uknes *et al*. 1993). PR1a in tobacco and *A. thaliana* is considered as a marker of the SA-dependent defence pathway SAR (Durner, Shah & Klessig 1997). In our studies, expression of the tomato isoform PR1a (P4) was up-regulated very rapidly following HHL and BHL application within the first 5 h, and more slowly after the addition of SA. This may be a result of the adsorption of SA to the soil substrate, thereby lowering its active concentration. Genes encoding other PR proteins that were induced by AHL were chitinases and an acidic β-1,3-glucanase. In tomato, acidic and basic isoforms of chitinases are differentially regulated. The 26 kDa acidic chitinase is induced by SA, whereas the 30 kDa basic chitinase is considered ethylene-dependent because it responds to ethephon, an ethylene-releasing compound (Van Kan *et al*. 1995).

Our results suggest that AHL molecules play an important role in the biocontrol activity of *S. liquefaciens* and other rhizobacteria in tomato, and act as mediators of communication between prokaryotes and eukaryotes. In line with these findings, Mathesius *et al*. (2003) reported specific responses of more than 150 proteins in roots of *M. truncatula* exposed to nM–μM micromolar concentrations of long chain (C12 to C16) AHL molecules. Even though SA contents were up-regulated systemically by inoculation with AHL-producing rhizobacteria, the pattern of AHL-induced defence genes was not confined to salicylate-dependent genes. In addition, the accumulation of specific transcripts depended on the AHL species. Hence, it may be speculated that tomato plants are able to perceive AHL molecules with yet unidentified receptor-based recognition mechanisms.

Experiments on producing AHLs using transgenically modified plants showed different effects on susceptibility after challenging tobacco and potato plants with soft-rot erwiniae (Mäe *et al*. 2001; Toth *et al*. 2004). It was found that AHLs derived from modified plants are able to interfere with quorum sensing regulated traits of pathogenic bacteria. It would be interesting to analyse the resistance of these plants against other pathogens, which do not use AHL-dependent quorum sensing systems, and to investigate their signalling systems for systemic responses in order to find out whether or not these plants are more resistant to plant pathogens.

The AHL-induced resistance by *S. liquefaciens* MG1 in tomato plants differs from that induced by *P. fluorescens*

WCS417r (Van Loon *et al*. 1998) in several respects. Firstly, *P. fluorescens* WCS417r does not produce AHLs in detectable amounts. Secondly, ISR in *A. thaliana* is not accompanied by elevated levels of plant signalling molecules, whereas SA contents are systemically induced by *S. liquefaciens* MG1 and AHL molecules. Thirdly, root application of two AHL molecules led to marked systemic inductions of defence gene expression, especially of pathogenesis-related proteins, whereas gene expression of defence-related proteins is not markedly altered in response to ISR-inducing rhizobacteria (Van Loon *et al*. 1998). Preliminary results show that AHLs are also effective in systemic induction of pathogen resistance in barley (Uta von Rad *et al*., unpublished results). AHLs may therefore be considered as potential candidates for a new group of general elicitors for plant defence because they are produced in the tomato rhizosphere in effective amounts, induce SA systemically, lead to enhanced gene expression for typical defence-related proteins and result to increased resistance against fungal pathogens. Interactions of this AHL-induced resistance with ISR and SAR require further analysis in future studies.

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REFERENCES

Andersen J., Sternberg C., Poulsen L., Bjorn S., Givskov M. & Molin S. (1998) New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Applied Environmental Microbiology* **64,** 2240–2246.

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. *Molecular Plant Microbe Interactions* **15,** 1147–1156.

Bahnweg G., Schulze S., Möller E.M., Rosenbrock H., Langebartels C. & Sandermann H. (1998) DNA isolation from recalcitrant materials such as tree roots, bark, and forest soil for the detection of fungal pathogens by polymerase chain reaction. *Annals of Biochemistry* **262,** 79–82.

Bellés J.M., Garro R., Fayos J., Navarro P., Primo J. & Conejero V. (1999) Gentisic acid as a pathogen-inducible signal, additional to salicylic acid for activation of plant defenses in tomato. *Molecular Plant Microbe Interactions* **12,** 227–235.

Berg G., Roskot N., Steidle A., Eberl L., Zock A. & Smalla K. (2002) Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. *Applied and Environmental Microbiology* **68,** 3328–3338.

Bloemberg G.V. & Lugtenberg B.J.J. (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology* **4,** 343–350.

Böhm J., Hahn A., Schubert R., Bahnweg G., Adler N., Nechwatal J., Oehlmann R. & Obwald W. (1999) Real-time quantitative PCR: DNA determination in isolated spores of the mycorrhizal fungus *Glomus mossae* and monitoring of *Phytophthora infestans* and *Phytophthora citricola* in their respective host plants. *Journal of Phytopathology* **147,** 409–416.

Campbell R. & Greaves M.P. (1990) Anatomy and community structure of the rhizosphere. In *The Rhizosphere* (ed. J.M. Lynch), pp. 11–34. Wiley, Chichester, England.

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.

De Meyer G., Audenaert K. & Höfte M. (1999) *Pseudomonas aeruginosa* 7NSK2-induced systemic resistance in tobacco depends on *in planta* salicylic acid accumulation but is not associated with PR1a expression. *European Journal of Plant Pathology* **105,** 513–517.

Duijff B.J., Gianinazzi-Pearson V. & Lemanceau P. (1997) Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. *New Phytologist* **135,** 325–334.

Durner J., Shah J. & Klessig D.F. (1997) Salicylic acid and disease resistance in plants. *Trends in Plant Science* **2,** 266–274.

Eberl L. (1999) N-Acyl homoserinelactone-mediated gene regulation in Gram-negative bacteria. *Systematic and Applied Microbiology* **22,** 493–506.

Eberl L., Winson M.K., Sternberg C., Stewart G.S.A.B., Christiansen G., Chhabra S.R., Bycroft B.W., Williams P., Molin S. & Givskov M. (1996) Involvement of *N*-acyl-L-homoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. *Molecular Microbiology* **20,** 127–136.

Eberl L., Molin S. & Givskov M. (1999) Surface motility of *Serratia liquefaciens* MG1. *Journal of Bacteriology* **181,** 1703–1712.

Elasri M., Delorme S., Lemanceau P., Stewart G., Laue B., Glickmann E., Oger P.M. & Dessaux Y. (2001) Acyl-homoserine lactone production is more common among plant-associated *Pseudomonas* spp. than among soilborne *Pseudomonas* spp. *Applied and Environmental Microbiology* **67,** 1198–1209.

Felix G., Duran J.D., Volko S. & Boller T. (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant Journal* **18,** 265–276.

Gao M., Teplitski M., Robinson J.B. & Bauer D.W. (2003) Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. *Molecular Plant Microbe Interactions* **16,** 827–834.

Hartmann A., Lawrence J.R., Assmus B. & Schloter M. (1998) Detection of microbes by laser confocal microscopy. In *Molecular Microbial Ecology Manual* (eds A.D.L. Akkermans, J.D. van Elsas & F.J. de Bruijn), Chapter 4.1.10, pp. 1–34. Kluwer, Dordrecht, the Netherlands.

Labbate M., Queek S.Y., Koh K.S., Rice S.A., Givskov M. & Kjelleberg S. (2004) Quorum sensing-controlled biofilm development in *Serratia liquefaciens* MG1. *Journal of Bacteriology* **186,** 692–698.

Leone G. & Tonneijk A.E.G. (1990) Acute ozone exposure predispose *Phaseolus vulgaris* beans to *Botrytis cinerea*. *Netherlands Journal of Plant Pathology* **96,** 65–74.

Mäe A., Montesano M., Koiv V. & Palva E.T. (2001) Transgenic plants producing the bacterial pheromone *N*-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopatho-

gen *Erwinia carotovora*. *Molecular Plant Microbe Interactions* **14,** 1035–1042.

Mathesius U., Mulders S., Gao M., Teplitski M., Rolfe B.G. & Bauer D.W. (2003) Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proceedings of the National Academy of Sciences, USA* **100,** 1444–1449.

Mercado-Blanco J., van der Drift K.M.G.M., Olsson P.E., Thomas-Oates J.E., van Loon L.C. & Bakker P.A.H.M. (2001) Analysis of the *pmsCEAB* gene cluster involved in biosynthesis of salicylic acid and the siderophore pseudomonine in the biocontrol strain *Pseudomonas fluorescens* WCS374. *Journal of Bacteriology* **183,** 1909–1920.

Meuwly P. & Métraux J.P. (1993) Ortho-anisic acid as internal standard for the simultaneous quantitation of salicylic acid and its putative biosynthetic precursors in cucumber leaves. *Annals of Biochemistry* **214,** 500–505.

Persello-Cartieaux F. (2003) Tales from the underground: molecular plant–rhizobia interactions. *Plant, Cell and Environment* **26,** 189–199.

Pierson L.S. III, Wood D.W. & Pierson E.A. (1998) Homoserine lactone-mediated gene regulation in plant-associated bacteria. *Annual Reviews of Phytopathology* **36,** 207–225.

Pieterse C.M.J. & van Loon L.C. (1999) Salicylic acid-independent plant defence pathways. *Trends in Plant Science* **4,** 52–58.

Pieterse C.M.J., van Wees S.C.M., Hoffland E., van Pelt J.A. & van Loon L.C. (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 C.M.J., van Pelt J.A., van Wees S.C.M., *et al.* (2001) Rhizobacteria-mediated induced systemic resistance: triggering, signalling and expression. *European Journal of Plant Pathology* **107,** 51–61.

Press C.M., Wilson M., Tuzun S. & Kloepper J.W. (1997) Salicylic acid produced by *Serratia marcescens* 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. *Molecular Plant Microbe Interactions* **10,** 761–768.

Riedel K., Ohnesorg T., Krogfelt K.A., Hansen T.S., Omori K., Givskov M. & Eberl L. (2001) *N*-acyl-L-homoserine lactone-mediated regulation of the Lip secretion system in *Serratia liquefaciens* MG1. *Journal of Bacteriology* **183,** 1805–1809.

Ryu C.M., Farag M.A., Hu C.H., Reddy M.S., Kloepper J.W. & Paré P.W. (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiology* **134,** 1017–1026.

Sambrook J. & Russel D.W. (2001) *Molecular Cloning*. Cold Spring Harbour Laboratory Press, New York, USA.

Shirasu K. & Schulze-Lefert P. (2000) Regulators of cell death in disease resistance. *Plant Molecular Biology* **44,** 371–385.

Smith R.S., Fedyk E.R., Springer T.A., Mukaida N., Iglewski B.H. & Phipps R.P. (2001) Il-8 production in human lung fibroblasts and epithelial cells activated by the Pseudomonas autoinducer N-3-oxododecanoyl homoserine lactone is transcriptionally regulated by NF-kappa B and activator protein-2. *Journal of Immunology* **167,** 366–374.

Steidle A., Sigl K., Schuhegger R., *et al.* (2001) Visualization of *N*-acylhomoserine lactone-mediated cell-cell communication between bacteria colonizing the tomato rhizosphere. *Applied and Environmental Microbiology* **67,** 5761–5770.

Steidle A., Allesen-Holm M., Riedel K., Berg G., Givskov M., Molin S. & Eberl L. (2002) Identification and characterization of an *N*-acylhomoserine lactone-dependent quorum-sensing system in *Pseudomonas putida* strain IsoF. *Applied and Environmental Microbiology* **68,** 6371–6382.

Teplitski M., Robinson J.B. & Bauer W.D. (2000) Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Molecular Plant Microbe Interactions* **13,** 637–648.

Thimm O., Essigmann B., Kloska S., Altmann T. & Buckhout T.J. (2001) Response of Arabidopsis to iron deficiency stress as revealed by microarray analysis. *Plant Physiology* **127,** 1030–1043.

Thomma B.P.H.J. (2003) *Alternaria* spp.: from general saprophyte to specific parasite. *Molecular Plant Pathology* **4,** 225–236.

Toth I.K., Newton J.A., Hyman L.J., Lees A.K., Daykin M., Ortori C., Williams P. & Fray R.G. (2004) Potato plants genetically modified to produce *N*-acylhomoserine lactones increase susceptibility to soft rot *Erwiniae*. *Molecular Plant Microbe Interactions* **17,** 880–887.

Tuomainen J., Betz C., Kangasjärvi J., Ernst D., Yin Z.H., Langebartels C. & Sandermann H.J. (1997) Ozone induction of ethylene emission in tomato plants: regulation by differential accumulation of transcripts for the biosynthetic enzymes. *Plant Journal* **12,** 1151–1162.

Uknes S., Dincher S., Friedrich L., Negrotto D., Williams S., Thompson-Taylor H., Potter S., Ward E. & Ryals J. (1993) Regulation of pathogenesis-related protein-1a gene expression in tobacco. *Plant Cell* **5,** 159–169.

Van Kan J.A.L., Cozijnsen T., Danhash N. & de Wit P.J.G.M. (1995) Induction of tomato stress protein mRNAs by ethephon, 2,6-dichloroisonicotinic acid and salicylate. *Plant Molecular Biology* **27,** 1205–1213.

Van Loon L.C., Bakker P.A.H.M. & Pieterse C.M.J. (1998) Systemic resistance induced by rhizosphere bacteria. *Annual Reviews of Phytopathology* **36,** 453–483.

Van Wees S.C.M., Luijendijk M., Smoorenburg I., van Loon L.C. & Pieterse C.M.J. (1999) Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. *Plant Molecular Biology* **41,** 537–549.

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