AEM
Journals.ASM.org

Pseudomonas Strains Naturally Associated with Potato Plants Produce Volatiles with High Potential for Inhibition of *Phytophthora infestans*

Lukas Hunziker,[a] Denise Bönisch,[a] Ulrike Groenhagen,[b] Aurélien Bailly,[a] Stefan Schulz,[b] Laure Weisskopf[a]

Ecology of Noxious and Beneficial Organisms, Institute for Sustainability Sciences, Agroscope, Zurich, Switzerland[a]; Institute of Organic Chemistry, Technische Universität Braunschweig, Braunschweig, Germany[b]

Bacteria emit volatile organic compounds with a wide range of effects on bacteria, fungi, plants, and animals. The antifungal potential of bacterial volatiles has been investigated with a broad span of phytopathogenic organisms, yet the reaction of oomycetes to these volatile signals is largely unknown. For instance, the response of the late blight-causing agent and most devastating oomycete pathogen worldwide, *Phytophthora infestans*, to bacterial volatiles has not been assessed so far. In this work, we analyzed this response and compared it to that of selected fungal and bacterial potato pathogens, using newly isolated, potato-associated bacterial strains as volatile emitters. *P. infestans* was highly susceptible to bacterial volatiles, while fungal and bacterial pathogens were less sensitive. Cyanogenic *Pseudomonas* strains were the most active, leading to complete growth inhibition, yet noncyanogenic ones also produced antioomycete volatiles. Headspace analysis of the emitted volatiles revealed 1-undecene as a compound produced by strains inducing volatile-mediated *P. infestans* growth inhibition. Supplying pure 1-undecene to *P. infestans* significantly reduced mycelial growth, sporangium formation, germination, and zoospore release in a dose-dependent manner. This work demonstrates the high sensitivity of *P. infestans* to bacterial volatiles and opens new perspectives for sustainable control of this devastating pathogen.

During the last decade, it has become evident that bacteria communicate with other organisms through the emission of volatile compounds. Highly significant volatile-mediated effects of bacteria have been reported for various target organisms, including bacteria themselves (1–5), plants (5–9), and fungi (10–12). The research carried out to understand the nature of this volatile-mediated interaction of bacteria with plants and with other bacteria has focused so far on model organisms (e.g., *Arabidopsis thaliana* and *Escherichia coli*) and has enabled identification of some of the active compounds involved in the respective interactions, such as indole, 2,3-butanediol, dimethyl disulfide, hydrogen sulfide, and ammonia. The research on model organisms has also contributed to understanding of the mechanisms underlying the observed phenotypic changes of increased (13–15) or decreased (16, 17) plant biomass and increased antibiotic resistance in bacteria (2–4, 18).

As far as fungi are concerned, most studies investigating their response to bacterial volatiles have focused on potential application and have thus largely neglected deeper investigation of the chemical nature of the active compounds and/or of the mode of action of these molecules. In addition to the inorganic volatiles hydrogen cyanide (19) and ammonia (20), few volatile organic compounds, such as sulfur compounds and long-chain ketones, have been unequivocally shown to inhibit the growth of phytopathogenic fungi when applied at biologically relevant concentrations (12). With the ultimate prospect of using the antifungal potential of bacterial volatiles for crop protection, most studies investigating fungal response to bacterial volatiles have used phytopathogenic fungi of agronomical relevance, such as *Fusarium*, *Rhizoctonia*, *Verticillium*, and many others (12, 21). Remarkably, oomycetes, a group of organisms phylogenetically related to algae but morphologically and physiologically close to fungi, have largely been ignored in previous studies that characterized the response of phytopathogenic organisms to bacterial volatiles, despite the heavy losses they cause in a wide range of crops (22, 23). For instance, the late blight-causing agent, *Phytophthora infestans*, which is responsible for the most devastating disease of potato worldwide, has so far not been tested for sensitivity to bacterial volatiles. The aims of this work were (i) to investigate the response of the oomycete *P. infestans* to bacterial volatiles, (ii) to compare this response to that of other selected potato pathogens belonging to different kingdoms (fungi and bacteria), and (iii) to identify active molecules responsible for the observed effects. To achieve these aims, we isolated a collection of bacterial strains from the rhizosphere and the phyllosphere of field-grown, late-blight-infested potato plants and assessed their volatile-mediated effects on the oomycete. We compared the response of *P. infestans* to that of other potato pathogens belonging to fungal and bacterial taxa, including *Rhizoctonia solani*, *Helminthosporium solani*, *Fusarium oxysporum*, and *Dickeya dianthicola*. Moreover, we analyzed the volatile profiles of a selected subset of strains to elucidate the chemical nature of the molecules responsible for the observed effects.

Received 15 September 2014 **Accepted** 11 November 2014

Accepted manuscript posted online 14 November 2014

Citation Hunziker L, Bönisch D, Groenhagen U, Bailly A, Schulz S, Weisskopf L. 2015. *Pseudomonas* strains naturally associated with potato plants produce volatiles with high potential for inhibition of *Phytophthora infestans*. Appl Environ Microbiol 81:821–830. doi:10.1128/AEM.02999-14.

Editor: D. Cullen

Address correspondence to Laure Weisskopf, laure.weisskopf@agroscope.admin.ch.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.02999-14.

doi:10.1128/AEM.02999-14

MATERIALS AND METHODS

Chemicals and culture media. Chemicals were purchased from Sigma-Aldrich (Switzerland) unless otherwise specified. Luria-Bertani (LB) medium was prepared by dissolving 20 g liter^{-1} of Difco LB broth, Lennox (BD), and adding 15 g liter^{-1} agar (Agar-agar; ERNE surface AG). For 10-fold-diluted LB (1/10 LB), 2 g liter^{-1} of LB broth was used. Actinomycete isolation agar (AMA) contained 22 g liter^{-1} Difco actinomycete isolation agar (BD) and 5 ml liter^{-1} glycerol (Sigma-Aldrich). Rye agar (RA) was prepared by simmering 200 g rye grains (winter rye cv. Picasso95) in 1.5 liters of tap water for ca. 1 h. The liquid was then filtered through a sieve (1.5-mm mesh) and made up to a final volume of 1 liter with tap water. Agar (20 g liter^{-1}) was added. Malt agar (MA) contained 15 g liter^{-1} Difco malt extract agar (BD) and 12 g liter^{-1} agar. Potato dextrose agar (PDA) was used at 39 g liter^{-1} (Oxoid). Petri dishes were filled using a plate-pouring machine (Mediajet; Integra Biosciences) with 18 ml of medium for standard petri dishes (94 by 16 mm; Greiner Bio-One) and 5.6 ml of medium for small dishes (55 by 14.2 mm; Gosselin, Semadeni). Medium for two-compartment petri dishes (94 by 15 mm; Greiner Bio-One) was poured manually, aiming at ca. 10 ml per compartment.

Fungal strains and culture conditions. A *P. infestans* polyspore isolate obtained in 2001 (provided by H. Krebs, Agroscope) was used for all experiments. This isolate had been maintained as a mycelial culture on RA supplemented with 5 g liter^{-1} D-glucose and regularly transferred to potato slices for host passages. The fungi *Rhizoctonia solani*, *Helminthosporium solani*, and *Botrytis cinerea* were obtained from P. Frei (Agroscope). A *Fusarium oxysporum* strain was isolated from infected tubers in 2013. The fungi were kept in 25% glycerol at −80°C and routinely grown on either MA (*R. solani* and *B. cinerea*) or PDA (*R. solani* and *H. solani*). Petri dishes were sealed with Parafilm M (Bemis flexible packaging) and stored in the dark at ca 20°C (fungi) or 18°C (*P. infestans*).

Bacterial strains and culture conditions. Most bacteria were newly isolated (see below). In addition to these newly isolated strains, we used *Pseudomonas protegens* CHA0 and CHA77, as well as *Pseudomonas chlororaphis* MA 342 (commercially available as Cerall), which were kindly provided by C. Keel and K. Lapouge from the University of Lausanne. *Dickeya dianthicola* strain 88-23 was kindly provided by S. Schaerer (Agroscope). Bacterial strains were routinely grown on LB and kept at −80°C in 25% glycerol for long-term storage.

Isolation of bacteria from field-grown potato plants. In October 2012, three potato plants were collected with their root systems and adhering soil from a field that had been previously artificially inoculated with *P. infestans* (experimental field from Agroscope, site Zurich-Reckenholz). For each of the three collected plants, leaves and stems (referred to here as "shoots") as well as root tissues were separated and treated as follows: shoots were ground in a disinfected ceramic mortar using ca. 5 ml of sterile water; roots and adhering soil were shaken in sterile water to collect the rhizosphere soil, while the roots themselves were discarded. The samples were homogenized by shaking and pipetted into a test tube with a cut tip. These suspensions were 10-fold serially diluted in sterile water and plated on three different isolation media: 1/10 LB, MA, and AMA (see above). Dilutions of 10^{-2} to 10^{-7} were plated on 1/10 LB and MA, and dilutions of 0 to 10^{-5} were plated on the selective medium AMA. All plates were incubated at ca. 20°C for at least 6 days. In order to cover as much of the cultivable diversity as possible, single colonies with different morphologies (per plant and sample type [rhizosphere versus phyllosphere]) were picked for isolation. A total of 137 bacterial strains were isolated from the different plants (79 from rhizosphere soil and 58 from the phyllosphere). Strains were then transferred to 1/10 LB and kept on this medium. A minority of the strains could not be grown in liquid culture and were harvested from LB plates to be resuspended in 0.9% NaCl. They were then treated in a manner similar to that used for the liquid LB cultures.

Phylogenetic identification of the strains using 16S and *rpoD* gene sequencing. Actively growing cells were picked with a sterile toothpick, which was vigorously stirred in a tube containing 50 μl of lysis buffer (50 mM KCl, 0.1% Tween 20, 10 mM Tris-HCl [pH 8.3]). After 99°C incubation for 10 min, 1 μl of the cell lysate was used as the template for PCR. Reaction mixtures contained 1× Go *Taq* Flexi buffer (Promega), 3 mM $MgCl_2$, 0.2 mM concentrations of deoxynucleoside triphosphates (dNTPs), a 0.4 μM concentration of each primer, and 1 U Go *Taq* Flexi polymerase. The universal 16S rRNA primers F (5′-AGAGTTTGATYMTGGCTCAG-3′; *E. coli* 16S rRNA gene positions 8 to 27; forward primer) and R (5′-CAKAAAGGAGGTGATCC-3′; *E. coli* 16S rRNA gene positions 1529 to 1545; reverse primer) were used (24). The PCR protocol included an initial denaturing step of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C, and 90 s at 72°C and a final elongation step of 10 min at 72°C. The reaction was performed with a thermocycler iCycler (Bio-Rad). Correct and specific amplification was verified by gel electrophoresis, and samples showing a single 1.5-kb band were purified using a Nucleo-Spin gel and PCR cleanup kit (Macherey-Nagel), following the manufacturer's instructions. Thereafter, a sequencing PCR (total volume, 5 μl) was performed utilizing 2 μl of the purified PCR product as the template, either one of the primers used for initial PCR (5 μM), BigDye sequencing buffer (1×), and 1 μl of BigDye Terminator from the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). The sequencing PCR started with 10 s denaturation (96°C) followed by 25 cycles of 5 s at 50°C and 4 min at 60°C. PCR products were subsequently purified with a BigDye Terminator kit according to the manufacturer's instructions (Applied Biosystems). Twenty microliters of purified DNA (supernatant) was finally pipetted into a 96-well plate and analyzed using a Prism 3300xl genetic analyzer (Applied Biosystems). The obtained sequences were analyzed with BioEdit (Tom Hall, Ibis Biosciences). The BLAST (basic local alignment search tool; http://blast.ncbi.nlm.nih.gov) interface and databank were used for identification of the strains to the genus level (see Table S1 in the supplemental material). Active strains affiliated with the genus *Pseudomonas* were characterized further using *rpoD* sequencing. To this end, a similar PCR protocol was applied as for the 16S PCR, with the following modifications: the primers used were rpoDf (5′-ACTTCCCTGGCACGGTTGACCA-3′) and rpoDr (5′-TCGACATGCGACGGTTGATGTC-3′) (25), the annealing temperature was 60°C, and the elongation time was 1 min. Purification, sequencing, and BLAST searching were performed as described above, and the results are shown in Table S2 in the supplemental material.

Dual-culture experiments with selected active strains. Of the 92 strains identified, 32 caused mycelial growth inhibition in a prescreen using *P. infestans*, *R. solani*, and *B. cinerea* as target organisms and were selected for further investigation. These 32 candidate strains were tested for antioomycete, antifungal, and antibacterial activity together with three well-characterized *Pseudomonas* strains used as controls (*P. protegens* CHA0, its isogenic cyanide mutant CHA77, and *P. chlororaphis* MA342). Five different target organisms were used: the oomycete *P. infestans*, the fungi *R. solani*, *H. solani* and *F. oxysporum*, and the bacterium *D. dianthicola*. Split petri dishes were used to analyze the volatile-mediated effect of the isolates on the target organisms. LB was poured on one half (isolated strains) and the respective medium (RA for *P. infestans* and *F. oxysporum*, PDA for *H. solani*, and *R. solani* or LB for *D. dianthicola*) on the other half (targets). To take the different growth speeds into account, the targets were inoculated at different time points: on the same day as bacteria (*D. dianthicola* and *R. solani*), 1 day before (*P. infestans*), 4 days before (*F. oxysporum*), or 14 days before (*H. solani*). Three drops of 10 μl of overnight bacterial culture adjusted to an optical density at 600 nm (OD_{600}) of 1 were spotted on one half of the split petri dish (LB for control plates), while the targets (a plug of mycelium or a drop of overnight culture for *D. dianthicola*) were inoculated on the other half. Plates were sealed with Parafilm, incubated at 20°C in the dark and photographed after 3 days for *R. solani*, 7 days for *D. dianthicola* and *F. oxysporum*, 14 days for *P. infestans*, and 28 days for *H. solani*. The plates were photographed from below with a reflex camera mounted on a stand. The obtained pictures were analyzed with the digital imaging software ImageJ (http://imagej.nih.gov/ij/). The mycelium area was determined with the freehand (or circle, for

D. dianthicola) area measurement tool of ImageJ. Growth was determined by subtracting the initial mycelial surface from that obtained after the given incubation time. This growth value was then compared to that measured in control plates (targets exposed to LB only), and a percentage was calculated. For *D. dianthicola*, integrated density (pixels) was used as a measure of colony density. These dual culture assays were performed with 3 or 4 biological replicates (petri dishes) per strain and target.

Analysis of HCN and NH_3 emission. The emission of volatile hydrogen cyanide (HCN) was detected by inoculating the candidate strains as one 10-μl drop (OD_{600} = 1) in a compartment of a split petri dish containing LB and placing a sterile piece of filter paper (1- by 1-cm^2 cuts) soaked in 5 mg/ml copper(II) ethyloacetate (Strem Chemicals, Newburyport, MA, USA) and 5 mg/ml 4,4-methylenebis-*N*,*N*-dimethylaniline (Fluka, Switzerland) in chloroform, which was air dried for 2 to 3 min, in the other compartment (left empty). *Pseudomonas protegens* strain CHA0 was used as a positive control, and its isogenic cyanide mutant CHA77 and LB were used as negative controls. After 1 day, filter papers were checked. A blue color indicated cyanide production. Ammonia was detected using the same split petri dish assay (LB on one side and nothing on the other) and a commercially available colorimetric reaction kit (MQuant Ammonium [NH_4^+] test; Merck, Darmstadt, Germany).

Collection of volatiles and GC-MS analysis. The volatiles of eight selected strains were collected and analyzed by gas chromatography-mass spectrometry (GC-MS) using closed-loop-stripping analysis (CLSA) as described earlier (5). The strains as well as uninoculated medium, as a control, were pregrown at 30°C in 6 ml LB liquid medium for 24 h. LB agar plates were inoculated with 300 μl of the preculture, cultivated for 24 h at 30°C, and then analyzed by CLSA at room temperature as described in reference 26. For each strain, three biological replicates were analyzed. In this system, air is continuously pumped (MB-21E; Senior Flexonics, Bartlett, IL) through the closed system containing an activated charcoal filter (precision charcoal filter, 5 mg; Chromtech GmbH, Idstein, Germany) and the agar plate for 24 h. Trapped volatiles were extracted from the charcoal filter by rinsing the filter three times with 15 μl dichloromethane (≥99.8%; Merck, Germany). The headspace extracts were subsequently analyzed by GC-MS. Media were analyzed without inoculation as control. GC-MS analyses were performed on a HP7890A GC connected to a HP5975C mass selective detector fitted with an HP-5ms fused silica capillary column (30 m; 0.22-mm inside diameter [i.d.]; 0.25-μm film; Agilent Technologies, USA). Conditions were as follows: inlet pressure, 67 kPa; He, 23.3 ml/min; injection volume, 1 μl; transfer line, 300°C; injector, 250°C; electron energy, 70 eV. The gas chromatograph was programmed as follows: 5 min at 50°C, then increasing 5°C/min to 320°C. Linear retention indices were determined from a homologous series of *n*-alkanes (C_8 to C_{32}). Compounds were identified by comparison of mass spectra to database spectra (from the Wiley Registry of Mass Spectral Data [7th ed.], NIST MS Library [2008 edition], and our own database created from synthesized reference compounds) and by comparison of the retention index data to standards (our own database and NIST Chemistry WebBook [http://webbook.nist.gov/chemistry; accessed 2013]).

Effect of 1-undecene on *P. infestans* mycelial growth and sporulation. The effect of pure 1-undecene and undecane on mycelial growth and sporangium formation of *P. infestans* was assessed as follows. Five-millimeter agar plugs from a growing mycelium were placed facing downward in the center of new RA plates. Silicone septa (5 mm, GR-2; Supelco) were soaked with definite quantities of the test compounds and placed in the center of the petri dish lid. Plates were sealed with Parafilm M and stored upside down in the dark at 18°C for 10 days. Mycelial growth was monitored by taking photographs and further analyzed using ImageJ. At the end of the incubation period, the plates were opened and sporangia were collected through the half cross-section of the plate by applying clear adhesive tape to the mycelium. Samples were directly mounted on glass slides, and snapshots were taken under the microscope every 3 mm from the center of the plate throughout the cross-section. Total numbers of closed and open sporangia were assessed using ImageJ. To assess the effect of pure 1-undecene on zoospore release, sporangia were collected from 1-week-old mycelial plates and pipetted onto 30-μl 0.2% agar drops containing various concentrations of 1-undecene. After 24 h of incubation at 4°C to trigger zoospore release from sporangia, closed, open, and germinating sporangia were counted. To investigate the effect of 1-undecene on direct sporangium germination (rather than zoospore release), the sporangium suspensions were vortexed for 30 s and amended with definite amounts of 1-undecene. They were then incubated for 30 min at room temperature with gentle agitation. The suspensions were then vortexed for another 30 s before being plated on 1.5% water-agar. The number of closed, open, and germinating sporangia were counted after 24 h incubation at 20°C.

TABLE 1 Taxonomic identity and antagonistic activity of 92 isolated strains[a]

Phylum	Genus	No. of strains in: Rhizosphere Total	Rhizosphere Active	Phyllosphere Total	Phyllosphere Active
Actinobacteria	*Agromyces*	0	0	1	1
	Arthrobacter	9	2	9	0
	Curtobacterium	0	0	4	2
	Frigoribacterium	0	0	2	0
	Microbacterium	6	1	6	0
	Nocardioides	1	0	0	0
	Plantibacter	1	0	3	0
	Rathayibacter	0	0	1	0
	Rhodococcus	2	1	0	0
	Streptomyces	3	0	1	1
Bacteroidetes	*Flavobacterium*	1	1	0	0
Firmicutes	*Bacillus*	7	3	1	0
	Sporosarcina	1	1	0	0
Proteobacteria	*Acidovorax*	1	0	0	0
	Citrobacter	1	0	0	0
	Enterobacter	2	0	0	0
	Janthinobacterium	1	0	0	0
	Methylobacterium	0	0	1	0
	Pseudomonas	14	10	10	9
	Rahnella	0	0	1	0
	Variovorax	2	0	0	0
Total		52	19	40	13

[a] Fifty-two bacterial strains isolated from the rhizosphere and 40 bacterial strains isolated from the phyllosphere of field-grown potato plants were identified to the genus level by sequencing of the 16S rRNA gene. A first screen for mycelial growth inhibition of *P. infestans*, *R. solani*, and *B. cinerea* enabled the selection of 32 candidate antagonistic strains (see Table S1 in the supplemental material). Strains were considered active when they reduced mycelial growth of at least one target pathogen by more than 25%.

Data analysis. Data were analyzed using the GraphPad Quikcalcs tools (http://www.graphpad.com/quickcalcs/), GraphPad Prism 5 software, and Microsoft Excel software.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in GenBank under accession numbers KP067092 to KP067180 for 16S sequences and KP067181 to KP067198 for *rpoD* sequences.

RESULTS

Isolates with volatile-mediated antagonistic activity. Isolation of morphologically distinct bacteria from potato plants on three

TABLE 2 Volatile-mediated activity of 32 strains isolated from rhizosphere (R) or phyllosphere (S) of field-grown potato plants[a]

Strain	Phylogenetic affiliation	Growth (% of control)					HCN emission	NH_3 concn (mg liter^{-1})
		P. infestans	*H. solani*	*R. solani*	*F. oxysporum*	*D. dianthicola*		
R32	*P. vranovensis*	**0**	**53**	76	88	**41**	+	**55**
R84	*P. marginalis*	**0**	**40**	**87**	97	**37**	+	ND
R01	*P. moraviensis*	**0**	**33**	92	100	**39**	+	ND
R47	*P. chlororaphis*	**0**	**59**	96	99	**38**	+	**35**
R82	*P. marginalis*	**0**	71	**73**	102	**53**	+	ND
R76	*P. fluorescens*	**13**	**50**	**69**	98	**70**	ND	**55**
S50	*P. moraviensis*	**3**	**39**	109	98	**52**	+	ND
S49	*P. fluorescens*	**3**	**55**	104	101	**56**	+	ND
R29	*Bacillus* sp.	**32**	**39**	91	101	99	ND	ND
S04	*P. frederiksbergensis*	**58**	77	**59**	101	77	ND	**205**
S24	*P. frederiksbergensis*	**21**	**70**	**86**	99	100	ND	**105**
R74	*P. frederiksbergensis*	**35**	79	**77**	99	105	ND	**355**
S35	*P. marginalis*	**30**	72	90	97	113	ND	ND
R02	*P. veronii*	**61**	93	**82**	96	91	ND	ND
S06	*P. frederiksbergensis*	**49**	**81**	**77**	**98**	121	ND	ND
R75	*P. frederiksbergensis*	**15**	73	91	**96**	**151**	ND	**35**
S27	*Arthrobacter* sp.	**50**	86	95	**97**	106	ND	**35**
R96	*Flavobacterium* sp.	**57**	90	98	101	93	ND	**255**
R31	*Sporosarcina* sp.	**82**	**56**	97	101	106	ND	ND
R60	*Arthrobacter* sp.	**75**	**59**	98	103	113	ND	ND
R95	*P. lini*	87	**60**	92	99	121	ND	**55**
R42	*Microbacterium* sp.	77	**39**	102	101	145	ND	ND
S25	*Curtobacterium* sp.	96	100	**82**	101	91	ND	ND
S19	*P. frederiksbergensis*	**62**	**63**	**83**	98	**164**	ND	ND
R61	*Arthrobacter* sp.	**66**	**72**	**115**	100	123	ND	**15**
S22	*P. syringae*	**86**	**42**	110	101	**136**	ND	ND
R54	*Bacillus* sp.	64	85	108	98	123	ND	ND
S34	*P. jessenii*	76	87	98	97	121	ND	ND
S01	*Streptomyces* sp.	98	**65**	95	101	133	ND	**205**
R73	*Bacillus* sp.	**79**	80	109	101	128	ND	**35**
S46	*Curtobacterium* sp.	108	**62**	103	103	128	ND	ND
R85	*Rhodococcus sp*	**75**	89	96	99	**181**	ND	ND
Cerall	*P. chlororaphis*	**2**	**69**	92	99	**51**	+	nt
Cha0	*P. protegens*	**2**	**50**	**76**	**95**	**42**	+	ND
Cha77	*P. protegens*	**68**	80	**77**	**93**	**200**	ND	ND

[a] Strains were tested against the oomycete *P. infestans*, the fungi *H. solani*, *R. solani*, and *F. oxysporum*, and the phytopathogenic bacterium *D. dianthicola*. Growth values are averages for 3 ot 4 replicates, expressed as a percentage of the control value. Bold values are significantly different from the control according to Student's *t* test (see Table S3 in the supplemental material for standard errors and significance levels). ND, not detected; nt, not tested. Strains are ordered according to their overall activity (most active first). Reference *Pseudomonas* strains (*P. chlororaphis* MA 342 [Cerall] and *P. protegens* CHA0 and its isogenic HCN-deficient mutant CHA77) are included for comparison.

different media yielded 137 strains, of which 92 could be phylogenetically identified to the genus or species level. A first screen of their putative antifungal activity yielded 32 isolates of interest (see Table S1 in the supplemental material). *Pseudomonas* strains were the most frequent among the identified isolates, and this genus also contained the highest proportion of active isolates (Table 1). In contrast, only two of 18 identified *Arthrobacter* and one of 12 identified *Microbacterium* strains showed antagonistic activity. These 32 active candidate strains were selected for further investigation to compare their volatile-mediated effects on five different potato pathogens.

Susceptibility to bacterial volatile and diffusible compounds varied greatly between the different targets, *F. oxysporum* being generally the least affected and *P. infestans* the most susceptible target organism (Table 2). Strikingly, *P. infestans* was the only target whose growth was completely arrested when it was exposed to the volatiles of the five most active strains, all belonging to the genus *Pseudomonas*. Transferring mycelial plugs exposed to the volatiles of the *Pseudomonas* strain R47 to fresh plates did not allow the oomycete to resume growth, suggesting a lethal effect (data not shown). In addition to these producers of lethal volatiles, other, overall less active strains, such as the *Pseudomonas* strains S35 and R75, the *Arthrobacter* strain S27, and the *Flavobacterium* strain R96, emitted volatiles that caused highly significant mycelial growth reduction in *P. infestans* without affecting the other targets. In contrast, susceptibility to the volatiles of the *Bacillus* strain R29 was shared with *H. solani*. The growth of the latter fungus was specifically inhibited by the volatiles of a few members of the *Actinobacteria* that did not inhibit any other target, e.g., *Streptomyces* strain S01, *Curtobacterium* strain S46, and *Microbacterium* strain R42. The fast-growing species *R. solani* was in general little affected by the volatiles of the isolated strains: even the most strongly inhibiting strain, S04, still allowed the fungus to grow to almost 60% of its normal growth (Table 2). *F. oxysporum* proved extremely resistant to bacterial volatiles, with hardly any significant growth inhibition upon exposure to any of the 32 strains. The

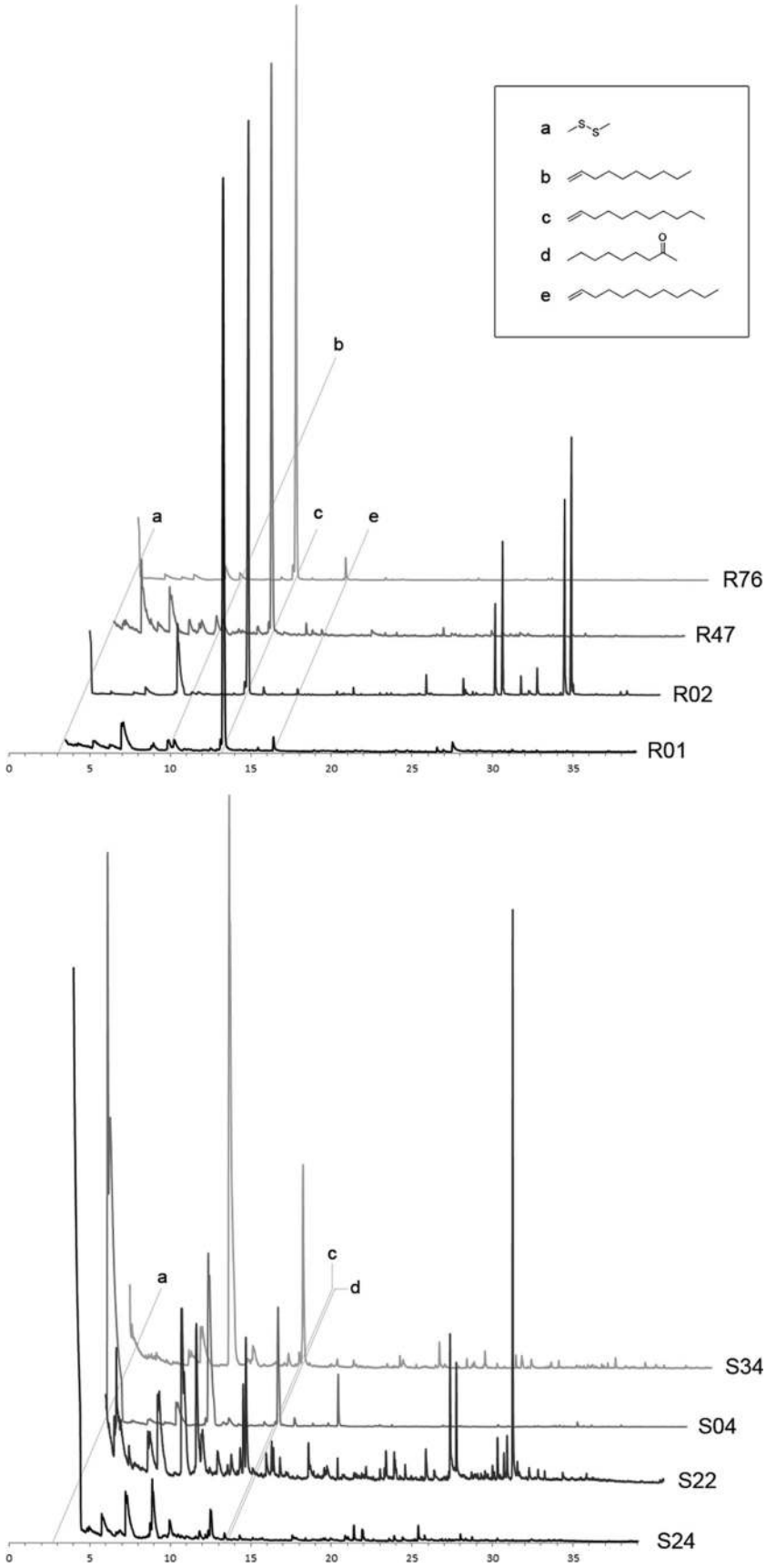

FIG 1 Overlays of total ion chromatograms of four rhizosphere (R) and four phyllosphere (S) *Pseudomonas* strains. Headspace sampling and GC-MS analysis were performed in triplicate, and one representative example is shown. The list of detected volatiles can be found in Table 3. The chemical structures of selected volatiles are shown. a, dimethyl disulfide; b, 1-decene; c, 1-undecene; d, 2-nonanone; e, 1-dodecene.

TABLE 3 Volatile compounds identified in the headspace of R01, R02, R47, R76, S04, S22, S24, and S34

RT (min)[a]	Compound[b]	*m/z*	*I* (exp.)[c]	*I* (lit.)[d]	Relative amt in[e]:							
					R01	R02	R47	R76	S04	S22	S24	S34
2.83	Dimethyl disulfide	94, 79, 45	774	777	x	x	x	xx	xxx	x	xxx	x
4.81	4-Hydroxy-4-methyl-2-pentanone	43, 59, 101, 39	844	842 (DB-5)		o				o		
5.22	2-Furanmethanol	39, 41, 98, 81	857	857	o		xx	o	o		o	
6.96	2,5-Dimethylpyrazine (A)	108, 42, 39	913	914	xx	o	x	o	x	o	x	x
7.10	2-Acetylfuran	95, 110, 38	916	916	o	o	x	o	o	o	o	o
8.18	Valine methyl ester	72, 55, 88	946				o					
8.76	Benzaldehyde	106, 77, 51	962	961		o				x		xxx
8.79	4-Methyl-4-butanolide	42, 56, 39, 100	963		o				o			
9.13	Dimethyl trisulfide	126, 79, 45, 64	971	972	o	xx	o	x	xx	x	x	xx
9.83	1-Decene	41, 55, 39	991	993	x			x				
9.84	*S*-Methyl butanethioate	43, 118, 75, 61	992							x		
10.22	Trimethylpyrazine (A)	42, 122, 39, 81	1,002	1,001	o	o	x	o	o	o	o	o
10.82	2-Acetylthiazole	43, 99, 58, 127	1,020	1,021	o	o			o		o	
11.40	Benzyl alcohol	79, 77, 108, 107	1,037	1,037						o		
12.40	*S*-Methyl methanethiosulfonate	45, 47, 63, 81	1,067	1,068			o	o		o		o
12.43	Acetophenone	77, 105, 51, 120	1,067	1,065	o	o	o		o	o		o
13.11	Tetramethylpyrazine (A)	54, 136, 42, 39	1,087	1,086			o		o	x	o	o
13.14	Undecadiene	41, 54, 67, 39	1,088		x	x		x			o	
13.28	1-Undecene	41, 55, 43, 56	1,092	1,092	xxx	xxx	xxx	xxx	x		o	
13.33	2-Nonanone	43, 58, 41, 57	1,094	1,093						x		x
13.38	Methyl benzoate	105, 77, 51, 136	1,095	1,094						x		
13.52	Methyl (*E*)-3-(methylthio)-2-propenoate	101, 45, 73, 58, 132	1,099							x		
14.31	Methyl (methylthio)methyl disulfide	61, 45, 140	1,124	1,123		o		o	o		o	
15.03	Unknown S-containing compound	57, 41, 120, 92	1,148							x		
16.06	Naphthalene	128, 102, 50	1,181	1,182						o		
16.40	1-Dodecene	41, 55, 43, 56	1,192	1,192	x	o		x				
16.47	2-Decanone	43, 58, 71	1,194	1,194								o
17.01	Dimethyl tetrasulfide	79, 45, 158	1,213	1,215					x			
18.17	Unknown S-containing compound	43, 71, 41, 134	1,254							x		
18.86	Tridecadiene	41, 67, 55, 81	1,278					o				
19.34	2-Undecanone	43, 58, 41	1,294	1,294								o
19.87	1,2-Epoxyundecane	41, 55, 71, 43	1,314	1,307		o						
23.50	Geranylacetone (A)	43, 41, 69, 67	1,454	1,455						o		
23.96	Unknown	43, 67, 41, 54	1,472							o	o	
24.38	Unknown N-containing compound	41, 97, 55, 43	1,490			o	o					
25.36	Dihydroactinidiolide	111, 109, 43	1,531	1,537						o		
26.83	Unknown N-containing compound	41, 55, 43, 97	1,592			o						
27.51	Diphenylamine (A)	169, 51, 65	1,624	1,622 (DB-5)	o	o		o		o	o	o
28.66	Unknown N-containing compound	41, 55, 122, 136	1,675			x						
28.70	Pentadec-8-en-2-one	43, 41, 55, 71	1,677							x		
29.13	Unknown N-containing compound	41, 43, 97, 55	1,696			x						
29.17	2-Pentadecanone	43, 58, 41	1,698	1,698						x		
31.27	Unknown N-containing compound	41, 43, 55, 97	1,796			x						
32.23	Hexahydrofarnesylacetone	43, 58, 41, 55	1,845	1,845						x		
32. 95	Heptadecen-2-one	43, 55, 41, 71	1,880							x		
32.98	Unknown N-containing compound	41, 55, 69, 122	1,881			xx						
33.36	2-Heptadecanone	43, 58, 41, 71	1,901	1,901						xx		
33.40	Unknown N-containing compound	41, 43, 55, 97	1,902			xx						
36.85	Unknown N-containing compound	55, 41, 69, 43	2,085			o						

[a] RT, retention time.
[b] Compounds were identified based on comparison of mass spectrum to a database spectrum, comparison of the retention index to a published retention index on the same or similar GC fused silica capillary column or comparison to a synthetic or commercially available reference compound. A, artifact (most likely a medium constituent).
[c] exp., experimental.
[d] lit., from the literature. Values were taken from the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry; accessed 2014) or our own database.
[e] Amounts are reported as being 0 to 1% (o), 1 to 10 % (x), 10 to 30 % (xx), and 30 to 100% (xxx) of the largest peak area in the total ion chromatogram.

phytopathogenic bacterium *D. dianthicola* was highly inhibited by the eight most active *Pseudomonas* strains (Table 2).

Emission of hydrogen cyanide and of other bioactive volatiles. Among the five target organisms tested, the oomycete and the bacterium responded more strongly to the volatile blends of the eight most active *Pseudomonas* strains than the three fungi (Table 2), suggesting greater sensitivity to the emitted volatiles. The capacity to emit the respiratory toxin hydrogen cyanide

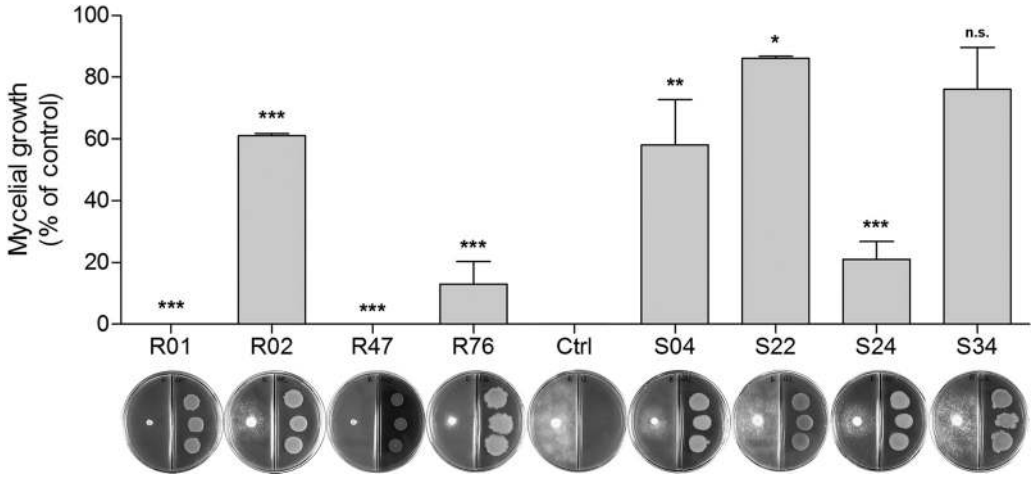

FIG 2 Volatile-mediated effects of four rhizosphere (R) and four phyllosphere (S) *Pseudomonas* strains on mycelial growth of *P. infestans*. Values are expressed as percentages of the value for the nonexposed control (100%). Significant differences from the control (according to Student's *t* test; $n = 3$ or 4) are indicated (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Representative pictures are shown; a picture of the nonexposed control (Ctrl) is shown in the middle between R strains (left) and S strains (right).

(HCN) was assessed in all strains and detected in seven of the eight most active *Pseudomonas* strains that triggered the highest inhibitory effects on both *P. infestans* and *D. dianthicola*. Comparing the effects of the volatiles from *P. protegens* CHA0 and from its isogenic cyanide-deficient mutant (CHA77) confirmed that inhibition of *D. dianthicola* was mostly, if not entirely, due to cyanide emission, given the growth promotion obtained upon exposure to the volatiles of CHA77 (Table 2).

In contrast, *P. infestans* was still significantly inhibited by the volatiles of the cyanide-deficient mutant, suggesting the emission of other volatiles with antioomycete potential. Emission of NH_3 was verified in the strains, and no correlation between the strains' antioomycete activity and their NH_3 production could be found (Table 2), suggesting that other compounds were responsible for the antioomycete activity of noncyanogenic strains. In order to elucidate the chemical nature of these compounds, the volatiles of eight *Pseudomonas* strains were collected and analyzed by GC-MS (Fig. 1; Table 3). These strains were chosen according to their origin of isolation (four from the rhizosphere and four from the phyllosphere) and to their global volatile-mediated activity, including highly active strains (R01, R47, and R76), moderately active ones (R02, S04, and S24), and practically inactive ones (S22 and S34) (Table 2). The volatile-mediated effects of these same eight *Pseudomonas* strains on *P. infestans* are depicted in Fig. 2. Of these strains, two produced HCN (R01 and R47), while the others did not. When the chromatograms of the eight selected strains were compared, a striking difference appeared between rhizosphere and phyllosphere strains, namely, the emission of massive amounts of 1-undecene in all four rhizosphere isolates (Fig. 1). These isolates were members of different species, as revealed by *rpoD* sequencing (see Table S3 in the supplemental material): *Pseudomonas moraviensis* (R1), *Pseudomonas veronii* (R2), *P. chlororaphis* (R47), and *P. fluorescens* (R76). In the phyllosphere isolates, smaller amounts of this compound were retrieved from the headspace of both strains identified as *Pseudomonas frederiksbergensis* (S04 and S24), while it was undetected in *Pseudomonas syringae* (S22) and in *Pseudomonas jessenii* (S34) (Table 3; Fig. 1). Few volatiles were commonly found in all investigated strains, such as the sulfur compounds dimethyl disulfide (most abundant in the two *P. frederiksbergensis* strains) and dimethyl trisulfide. 2-Acetylfurane was ubiquitous, while a group of nitrogen-containing compounds were found only in the headspace of *P. veronii* (R02), a rhizosphere strain of moderately antagonistic activity (Fig. 1 and 2; Table 3). The volatile profile of *P. syringae* S22 clearly differed from the seven others analyzed. This strain emitted less alkenes than the others, but a collection of ketones was detected in its headspace. Volatiles emitted mostly by strains showing antioomycete activity and less by those showing less antioomycete activity revealed dimethyl disulfide, 1-decene, 1-undecene, 1-dodecene, and an undecadiene with an unknown location of double bonds as putative mediators of antioomycete activity (Fig. 1; Table 3).

1-Undecene inhibits the mycelial growth of *P. infestans* and changes its sporulation behavior. Since 1-undecene was produced in very high quantities in all four rhizosphere strains and was also detected in the two phyllosphere strains with moderate but significant antioomycete activity (Fig. 2), we investigated whether this volatile might be involved in the observed mycelium growth inhibition of *P. infestans*. When different quantities of 1-undecene and, for a comparison, of undecane (which was not detected in the strains' headspace) were added to the *P. infestans* medium, a dose-dependent mycelium inhibition was observed for 1-undecene which was much stronger than that obtained with its reduced form, undecane (Fig. 3a). Moreover, the total amount of sporangia was significantly reduced in a concentration-dependent manner in 1-undecene treatments compared to the control treatment, as was the proportion of open sporangia, which had released the zoospores (Fig. 3b and c). Moreover, native (untreated) sporangia spotted on water agar drops containing increasing quantities of 1-undecene revealed that this molecule directly impacted zoospore release as well as direct germination of the sporangia (Fig. 3d). When applied in large amounts (from 0.75 mg on), the proportion of open sporangia was drastically reduced and the direct germination almost completely inhibited.

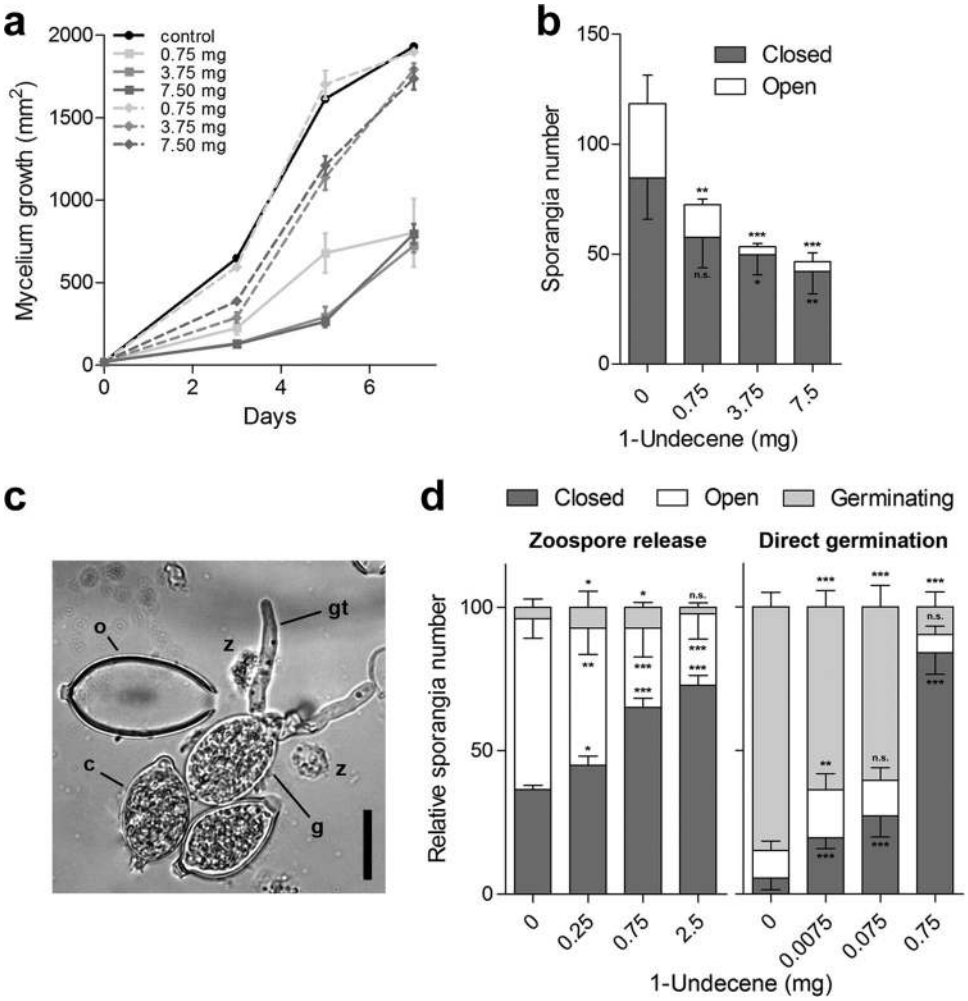

FIG 3 Growth and sporulation of *P. infestans* in the presence of volatile 1-undecene. (a) Mycelium growth in the presence of different quantities of 1-undecene (squares) or undecane (diamonds) applied as volatiles. Data points represent the average surface mycelium growth of four to six replicates, with standard deviation bars included. (b) Absolute number of closed and open sporangia at 3 mm from the mycelium origin under volatile 1-undecene exposure. Results are representative of biological duplicates and are expressed as means ± standard errors of the means (SEM). (c) Representative examples of closed sporangia (c) containing zoospores (z), open sporangia (o), and directly germinating sporangia (g) with the germ tube (gt). Bar = 10 μm. (d) Relative numbers of closed, open, and germinating sporangia (right, zoospore release; left, direct germination). Results shown are representative of biological triplicates and are expressed as means ± SEM. Asterisks indicate statistical significance according to one-way Analysis of variance (ANOVA) followed by Dunnett's *post hoc* test compared to control treatments ($n = 9$; $P > 0.001$). n.s., $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

DISCUSSION

Many fungal disease-causing agents have been evaluated for their reaction to bacterial volatiles, which are often highly target specific (summarized in reference 12). However, oomycetes have largely been ignored in those studies, although they include pathogens causing major losses in global crop production (22, 23). One example of such a pathogen is the late blight-causing agent *P. infestans*, which was responsible for the Great Famine of Ireland in the middle of the 19th century and is the most important potato disease worldwide. In an attempt to analyze the yet-unknown response of this pathogen to bacterial volatiles, we first isolated bacterial strains from an environment where they would have been likely to encounter the pathogen, namely, from the phyllosphere and rhizosphere of field-grown, late blight-infected potato plants. Since previous reports indicated emission of antifungal volatiles from *Actinobacteria* (27–29), we used, in addition to broad-spectrum media, an *Actinobacteria*-specific medium. We thus isolated many *Actinobacteria* strains (Table 1), but only a few of them (mostly *Arthrobacter* strains) induced high volatile-mediated inhibition of *P. infestans* or of the other targets, while *Pseudomonas* strains proved the most efficient producers of growth-inhibiting volatiles under our cultivation conditions (Table 2).

When the overall susceptibilities of our different targets to bacterial volatiles were compared, *P. infestans* was clearly the organism responding most strongly, followed by the silver scurf-causing fungus *H. solani* and the black leg-inducing bacterium *D. dianthicola*. This confirms earlier findings indicating high sensitivity of *Pythium* species or of other *Phytophthora* species to bacterial volatiles (21, 30). A substantial difference between fungi and oomycetes is the structure of their cell walls (chitin for fungi and cellulose for oomycetes). Although this has not yet been assessed, it is tempting to speculate that the cell wall composition and the struc-

ture of oomycetes would make them more permeable to gaseous substances than that of fungi and that this might at least partly account for the higher sensitivity of oomycetes to bacterial volatiles.

Most of our highly active isolates were cyanogenic *Pseudomonas* strains. The inhibitory potential of this respiratory toxin is not new, and neither is its involvement in the biocontrol efficiency of cyanogenic *Pseudomonas* strains (19, 31). However, different organisms might show different tolerance levels to hydrogen cyanide, as observed in this study: *P. infestans* and *D. dianthicola* reacted most strongly to volatiles from cyanogenic strains, while hydrogen cyanide showed lesser toxicity to the fungi tested and especially to *R. solani* and *F. oxysporum*, for which no difference in inhibition was observed between the model strains *P. protegens* CHA0 and its isogenic cyanide mutant CHA77 (Table 2).

Interestingly, our results show that noncyanogenic *Pseudomonas* also induced significant volatile-mediated growth reduction in *P. infestans*, suggesting the presence of other bioactive volatiles. The volatile profiles of the four rhizosphere *Pseudomonas* strains analyzed were dominated by the alkene 1-undecene, while this compound was undetected or present only in small quantities in the phyllosphere strains. In a meta-analysis of 31 studies on volatiles emitted by six common pathogenic bacteria, 1-undecene was one of the molecules that differentiated *Pseudomonas aeruginosa* from the other, non-*Pseudomonas* pathogenic bacteria (32). Within the genus *Pseudomonas*, it seems that the relative contribution of undecene to the volatile bouquet of the strains is highly variable, ranging from the unchallenged dominance observed in our rhizosphere strains or in other antagonistic *P. fluorescens* strains (33) to the absence of detection for some strains, such as *P. syringae* S22 and *P. jessenii* S34. Other studies have reported 1-undecene from the headspace of *Pseudomonas* strains (7, 34–36) but also from other species, including members of the genera *Burkholderia*, *Bacillus*, and *Serratia* (7, 37).

1-Undecene seemed a good candidate to explain the growth-inhibiting effects observed upon exposure of *P. infestans* to noncyanogenic strains, since it was emitted in large amounts and was mostly detected in the strains showing antioomycete activity. In addition to mycelial growth inhibition, the formation of sporangia and their zoospore release were also significantly impaired by exposure to 1-undecene. This is of particular interest considering the epidemiology of *P. infestans*: the pathogen undergoes multiple asexual reproductive cycles during a potato cropping season and the motile zoospores released from the sporangia are a key element in the infection of new leaf tissues and tubers. Preventing their release might thus have more significant consequences on the disease spread and on the harvest quantity and quality than solely reducing the oomycete's vegetative growth. Further studies will reveal to what extent 1-undecene is produced when the strains' growth conditions are closer to the field situation and what the actual potential in terms of potato protection is.

Very few studies have analyzed the volatile emission by strains grown directly on or in their host (33) or at least on substrates mimicking the strains' natural environment (7, 21). Overall, the results of these studies were encouraging, since they demonstrated that (i) the active volatile (dimethyl disulfide) detected in the headspace of the antagonist strain grown in *in vitro* cultures could be detected *in vivo* as well, i.e., when the strain was growing inside the plants (33), and (ii) that bacteria were able to maintain significant levels of activity even when supplied with very low levels of nutrients (7, 21).

Our isolation of bacteria from the rhizosphere and phyllosphere of potato yielded a collection of strains displaying a high potential for volatile-mediated inhibition of *P. infestans*. The present study shows that the oomycete is highly susceptible to the volatiles of cyanogenic and noncyanogenic strains and that the massively emitted alkene 1-undecene partly accounts for volatile-mediated growth inhibition of the late blight-causing agent. Further studies could on the one hand elucidate the mechanisms by which bioactive volatiles such as 1-undecene impede the oomycete's growth and on the other hand evaluate the potential of volatile-emitting antagonists for potato protection in experimental setups that are closer to the field situation.

ACKNOWLEDGMENTS

We are grateful to Daniel Marty and Anouk Guyer for excellent technical assistance, to Hans-Rudolf Forrer, Heinz Krebs, Tomke Musa, and Natacha Bodenhausen for helpful suggestions, and to Susanne Vogelgsang for proofreading the manuscript.

This research was partially financed by the Swiss National Science Foundation (grant 31003A-149271 to L.W.).

REFERENCES

1. **Chernin L, Toklikishvili N, Ovadis M, Kim S, Ben-Ari J, Khmel I, Vainstein A.** 2011. Quorum-sensing quenching by rhizobacterial volatiles. Environ Microb Rep **3:**698–704. http://dx.doi.org/10.1111/j.1758-2229.2011.00284.x.
2. **Letoffe S, Audrain B, Bernier SP, Delepierre M, Ghigo JM.** 2014. Aerial exposure to the bacterial volatile compound trimethylamine modifies antibiotic resistance of physically separated bacteria by raising culture medium pH. mBio **5:**e00944-13. http://dx.doi.org/10.1128/mBio.00944-13.
3. **Bernier SP, Letoffe S, Delepierre M, Ghigo JM.** 2011. Biogenic ammonia modifies antibiotic resistance at a distance in physically separated bacteria. Mol Microbiol **81:**705–716. http://dx.doi.org/10.1111/j.1365-2958.2011.07724.x.
4. **Shatalin K, Shatalina E, Mironov A, Nudler E.** 2011. H2S: a universal defense against antibiotics in bacteria. Science **334:**986–990. http://dx.doi.org/10.1126/science.1209855.
5. **Groenhagen U, Baumgartner R, Bailly A, Gardiner A, Eberl L, Schulz S, Weisskopf L.** 2013. Production of bioactive volatiles by *Burkholderia ambifaria* strains. J Chem Ecol **39:**892–906. http://dx.doi.org/10.1007/s10886-013-0315-y.
6. **Bailly A, Weisskopf L.** 2012. The modulating effect of bacterial volatiles on plant growth: current knowledge and future challenges. Plant Signal Behav **7:**79–85. http://dx.doi.org/10.4161/psb.7.1.18418.
7. **Blom D, Fabbri C, Connor EC, Schiestl FP, Klauser DR, Boller T, Eberl L, Weisskopf L.** 2011. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environ Microbiol **13:**3047–3058. http://dx.doi.org/10.1111/j.1462-2920.2011.02582.x.
8. **Meldau DG, Meldau S, Hoang LH, Underberg S, Wunsche H, Baldwin IT.** 2013. Dimethyl disulfide produced by the naturally associated bacterium *Bacillus* sp B55 promotes *Nicotiana attenuata* growth by enhancing sulfur nutrition. Plant Cell **25:**2731–2747. http://dx.doi.org/10.1105/tpc.113.114744.
9. **Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, Kloepper JW.** 2003. Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci U S A **100:**4927–4932. http://dx.doi.org/10.1073/pnas.0730845100.
10. **Campos VP, de Pinho RSC, Freire ES.** 2010. Volatiles produced by interacting microorganisms potentially useful for the control of plant pathogens. Cienc Agrotecnol **34:**525–535. http://dx.doi.org/10.1590/S1413-70542010000300001.
11. **Effmert U, Kalderas J, Warnke R, Piechulla B.** 2012. Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol **38:**665–703. http://dx.doi.org/10.1007/s10886-012-0135-5.
12. **Weisskopf L.** 2013. The potential of bacterial volatiles for crop protection

against phytopathogenic fungi, p 1352–1363. *In* Méndez-Vilas A (ed), Microbial pathogens and strategies for combating them: science, technology and education. Formatex, Badajoz, Spain.
13. **Zhang H, Kim MS, Krishnamachari V, Payton P, Sun Y, Grimson M, Farag MA, Ryu CM, Allen R, Melo IS, Pare PW.** 2007. Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. Planta **226:**839–851. http://dx.doi.org/10.1007/s00425-007-0530-2.
14. **Zhang HM, Sun Y, Xie XT, Kim MS, Dowd SE, Pare PW.** 2009. A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. Plant J **58:**568–577. http://dx.doi.org/10.1111/j.1365-313X.2009.03803.x.
15. **Zhang HM, Xie XT, Kim MS, Kornyeyev DA, Holaday S, Pare PW.** 2008. Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. Plant J **56:**264–273. http://dx.doi.org/10.1111/j.1365-313X.2008.03593.x.
16. **Blom D, Fabbri C, Eberl L, Weisskopf L.** 2011. Volatile-mediated killing of *Arabidopsis thaliana* by bacteria is mainly due to hydrogen cyanide. Appl Environ Microbiol **77:**1000–1008. http://dx.doi.org/10.1128/AEM.01968-10.
17. **Wenke K, Wanke D, Kilian J, Berendzen K, Harter K, Piechulla B.** 2012. Volatiles of two growth-inhibiting rhizobacteria commonly engage AtWRKY18 function. Plant J **70:**445–459. http://dx.doi.org/10.1111/j.1365-313X.2011.04891.x.
18. **Lee JH, Lee J.** 2010. Indole as an intercellular signal in microbial communities. FEMS Microbiol Rev **34:**426–444. http://dx.doi.org/10.1111/j.1574-6976.2009.00204.x.
19. **Voisard C, Keel C, Haas D, Defago G.** 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root-rot of tobacco under gnotobiotic conditions. EMBO J **8:**351–358.
20. **Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B.** 2009. Bacterial volatiles and their action potential. Appl Microbiol Biot **81:**1001–1012. http://dx.doi.org/10.1007/s00253-008-1760-3.
21. **Garbeva P, Hordijk C, Gerards S, de Boer W.** 2014. Volatiles produced by the mycophagous soil bacterium *Collimonas*. FEMS Microbiol Ecol **87:**639–649. http://dx.doi.org/10.1111/1574-6941.12252.
22. **Strange RN, Scott PR.** 2005. Plant disease: a threat to global food security. Annu Rev Phytopathol **43:**83–116. http://dx.doi.org/10.1146/annurev.phyto.43.113004.133839.
23. **Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ.** 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature **484:**186–194. http://dx.doi.org/10.1038/nature10947.
24. **Juretschko S, Timmermann G, Schmid M, Schleifer KH, Pommerening-Roser A, Koops HP, Wagner M.** 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. Appl Environ Microbiol **64:**3042–3051.
25. **Frapolli M, Defago G, Moenne-Loccoz Y.** 2007. Multilocus sequence analysis of biocontrol fluorescent *Pseudomonas* spp. producing the antifungal compound 2,4-diacetylphloroglucinol. Environ Microbiol **9:**1939–1955. http://dx.doi.org/10.1111/j.1462-2920.2007.01310.x.
26. **Schulz S, Fuhlendorff J, Reichenbach H.** 2004. Identification and synthesis of volatiles released by the myxobacterium *Chondromyces crocatus*. Tetrahedron **60:**3863–3872. http://dx.doi.org/10.1016/j.tet.2004.03.005.
27. **Li QL, Ning P, Zheng L, Huang JB, Li GQ, Hsiang T.** 2012. Effects of volatile substances of *Streptomyces globisporus* JK-1 on control of *Botrytis cinerea* on tomato fruit. Biol Control **61:**113–120. http://dx.doi.org/10.1016/j.biocontrol.2011.10.014.
28. **Wan MG, Li GQ, Zhang JB, Jiang DH, Huang HC.** 2008. Effect of volatile substances of *Streptomyces platensis* F-1 on control of plant fungal diseases. Biol Control **46:**552–559. http://dx.doi.org/10.1016/j.biocontrol.2008.05.015.
29. **Wang CL, Wang ZF, Qiao X, Li ZJ, Li FJ, Chen MH, Wang YR, Huang YF, Cui HY.** 2013. Antifungal activity of volatile organic compounds from *Streptomyces alboflavus* TD-1. FEMS Microbiol Lett **341:**45–51. http://dx.doi.org/10.1111/1574-6968.12088.
30. **Chaurasia B, Pandey A, Palni LMS, Trivedi P, Kumar B, Colvin N.** 2005. Diffusible and volatile compounds produced by an antagonistic *Bacillus subtilis* strain cause structural deformations in pathogenic fungi in vitro. Microbiol Res **160:**75–81. http://dx.doi.org/10.1016/j.micres.2004.09.013.
31. **Haas D, Defago G.** 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol **3:**307–319. http://dx.doi.org/10.1038/nrmicro1129.
32. **Bos LDJ, Sterk PJ, Schultz MJ.** 2013. Volatile metabolites of pathogens: a systematic review. PLoS Pathog **9:**e1003311. http://dx.doi.org/10.1371/journal.ppat.1003311.
33. **Dandurishvili N, Toklikishvili N, Ovadis M, Eliashvili P, Giorgobiani N, Keshelava R, Tediashvili M, Vainstein A, Khmel I, Szegedi E, Chernin L.** 2011. Broad-range antagonistic rhizobacteria *Pseudomonas fluorescens* and *Serratia plymuthica* suppress *Agrobacterium* crown gall tumours on tomato plants. J Appl Microbiol **110:**341–352. http://dx.doi.org/10.1111/j.1365-2672.2010.04891.x.
34. **Fernando WGD, Ramarathnam R, Krishnamoorthy AS, Savchuk SC.** 2005. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol Biochem **37:**955–964. http://dx.doi.org/10.1016/j.soilbio.2004.10.021.
35. **Kai M, Effmert U, Berg G, Piechulla B.** 2007. Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch Microbiol **187:**351–360. http://dx.doi.org/10.1007/s00203-006-0199-0.
36. **Schulz S, Dickschat JS.** 2007. Bacterial volatiles: the smell of small organisms. Nat Prod Rep **24:**814–842. http://dx.doi.org/10.1039/b507392h.
37. **Lee B, Farag MA, Park HB, Kloepper JW, Lee SH, Ryu C-M.** 2012. Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*. PLoS One **7:**e48744. http://dx.doi.org/10.1371/journal.pone.0048744.