Taylor & Francis
Taylor & Francis Group

Communicative & Integrative Biology

ISSN: (Print) 1942-0889 (Online) Journal homepage: http://www.tandfonline.com/loi/kcib20

Making healthier or killing enemies? Bacterial volatile-elicited plant immunity plays major role upon protection of Arabidopsis than the direct pathogen inhibition

Rouhallah Sharifi & Choong-Min Ryu

To cite this article: Rouhallah Sharifi & Choong-Min Ryu (2016) Making healthier or killing enemies? Bacterial volatile-elicited plant immunity plays major role upon protection of Arabidopsis than the direct pathogen inhibition, Communicative & Integrative Biology, 9:4, e1197445, DOI: 10.1080/19420889.2016.1197445

To link to this article: http://dx.doi.org/10.1080/19420889.2016.1197445

Accepted author version posted online: 12 Jul 2016.
Published online: 12 Jul 2016.

COMMUNICATIVE & INTEGRATIVE BIOLOGY
2016, VOL. 9, NO. 4, e1197445 (3 pages)
http://dx.doi.org/10.1080/19420889.2016.1197445

Taylor & Francis
Taylor & Francis Group

ARTICLE ADDENDUM

OPEN ACCESS

Making healthier or killing enemies? Bacterial volatile-elicited plant immunity plays major role upon protection of *Arabidopsis* than the direct pathogen inhibition

Rouhallah Sharifi[a] and Choong-Min Ryu[b,c]

[a]Department of Plant Protection, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran; [b]Molecular Phytobacteriology Laboratory, Superbacteria Research Center, KRIBB, Daejeon, South Korea; [c]Biosystem and Bioengineering Program, University of Science and Technology (UST), Daejeon, South Korea

ABSTRACT
Bacterial volatiles protect plants either by directly inhibiting a pathogenic fungus or by improving the defense capabilities of plants. The effect of bacterial volatiles on fungal growth was dose-dependent. A low dosage did not have a noticeable effect on *Botrytis cinerea* growth and development, but was sufficient to elicit induced resistance in *Arabidopsis thaliana*. Bacterial volatiles displayed negative effects on biofilm formation on a polystyrene surface and in *in planta* leaf colonization of *B. cinerea*. However, bacterial volatile-mediated induced resistance was the major mechanism mediating protection of plants from *B. cinerea*. It was responsible for more than 90% of plant protection in comparison with direct fungal inhibition. Our results broaden our knowledge of the role of bacterial volatiles in plant protection.

ARTICLE HISTORY
Received 18 May 2016
Revised 27 May 2016
Accepted 27 May 2016

KEYWORDS
bacterial volatile organic compounds; fungal inhibition; induced systemic resistance; leaf surface attachment; plant growth-promoting rhizobacteria

Bacteria emit a vast array of volatile organic compounds belonging to various chemical groups.[1] More than 120 individual volatiles have been identified in actinomycetes, a group of Gram-positive bacteria.[2] Based on this diversity, bacterial volatiles have different and even opposite biological activities in natural and artificial systems. They increase or inhibit the growth of fungi,[2-9] induce resistance to biotic[10-16] and abiotic[17] stresses, and promote[9,15] or suppress plant growth.[18]

Plant protection by bacterial volatiles is mediated by 2 distinct. First, bacterial volatiles are able to protect plants via inhibition of fungal growth and development. Volatiles from *Bacillus* spp. decreased pigmentation in *Fusarium oxysporum*[6] and *B. cinerea*.[8] In our previous work, we revealed that the effect of volatiles on *B. cinerea* was dose-dependent.[7] Exposure of fungi to bacterial volatiles from one colony of *B. subtilis* GB03 did not have a significant effect on fungal growth, spore production, and spore germination (Fig. 1). However, there was a linear relationship between the volatile concentration and fungal inhibition.[7] Quintana-Rodriguez and coworkers[10] also showed that volatiles emitted from the common bean plant were able to directly inhibit conidia germination *in vivo* and *in vitro* in a dose-dependent manner. Furthermore, bacterial volatiles had a negative effect on biofilm formation on a polystyrene surface in a dose-dependent manner.[7] It should be noted that some volatiles were able to increase mycelial growth and spore production and germination of different fungi. Volatiles of *Klebsiella pneumonia* increased growth and spore germination of the mycorrhizal fungus *Glomus mosseae*.[19] Acetoin, 2,3-butanediol, and 3-pentanol improved fungal growth and spore production of *B. cinerea* at a concentration of 100 μM.[8]

Secondly, volatiles can protect plants via induced systemic resistance (ISR) against pathogens. The long-chain volatiles tridecane[14] and hexadecane[15] induced resistance in *Arabidopsis* against *Pectobacterium carotovorum* and *Pseudomonas syringae*, respectively. A low dosage of butanediol suppressed *Microdochium nivale* in *Agrostis stolonifera* by up to 90%.[12] The same concentration of acetoin induced resistance against *P. syringae* pv. *tomato* in *Arabidopsis*.[16] In our previous work, volatiles of *B. subtilis* GB03 and 100 μM 2-hydroxy-3-pentanone suppressed the growth of *B. cinerea* on *Arabidopsis*.[8]

In our previous work,[7] we designed an experiment to determine the contribution of each mechanism, direct fungal inhibition or boosting of plant immunity, to protection of *Arabidopsis* against *B. cinerea*. We found that a low

CONTACT Choong-Min Ryu cmryu@kribb.re.kr Molecular Phytobacteriology Laboratory, Superbacteria Research Center, KRIBB, Daejeon 305-806, South Korea.
Addendum to: Sharifi R., Ryu C.-M. Are bacterial volatile compounds poisonous odors to a fungal pathogen Botrytis cinerea, alarm signals to Arabidopsis seedlings for eliciting induced resistance, or both? Front Microbiol 2016; 7:196; http://dx.doi.org/10.3389/fmicb.2016.00196

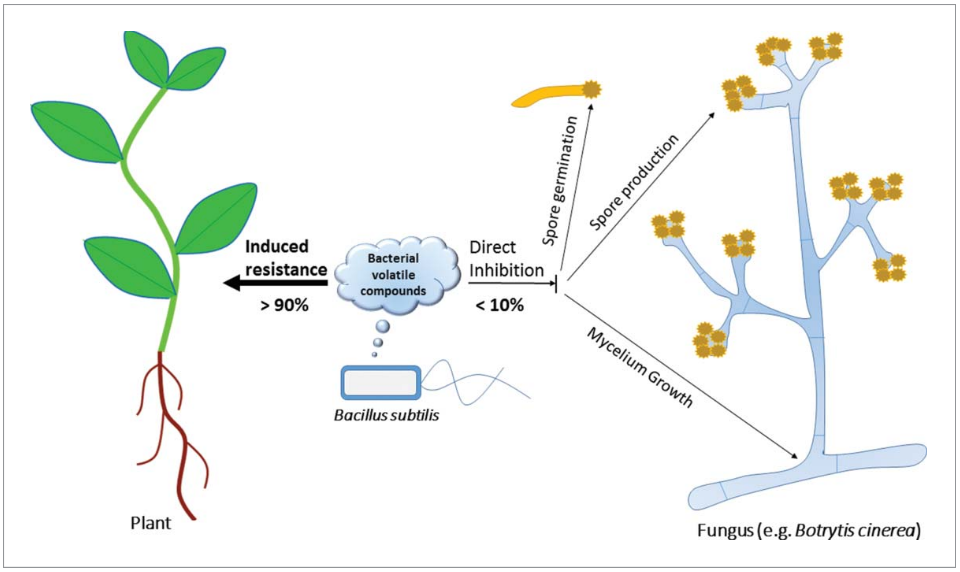

Figure 1. Illustrated model of the role of bacterial volatile compounds (BVCs) in plant protection against pathogenic fungi. The major mechanism for plant protection by BVCs from the soil bacterium *Bacillus subtilis* is induced resistance (responsible for more than 90% of plant protection). BVCs attenuate mycelial growth, spore production, and spore germination of fungi including *Botrytis cinerea* when used at high dosages, but this direct effect is responsible for less than 10% of plant protection when the optimum dosage is used.

concentration of volatiles was sufficient to elicit induced resistance in plants, but was not sufficient to inhibit fungal growth and development.[7] ISR and direct fungal inhibition were responsible for more than 90% and less than 10% of plant protection, respectively (Fig. 1).[7] Microscopic inspection showed that a low dose of volatiles affected leaf colonization of *B. cinerea* by increasing epiphytic growth of the fungus, but this effect was unstable.

Volatiles of *B. subtilis* GB03 primed the expression of *PR1* and *PDF1.2*, but not of *ChiB*, indicating activation of a salicylic acid (SA)- and jasmonic acid (JA)-dependent signaling pathways. However, the ISR signaling pathways could differ based on the profile of volatiles released by different bacteria. For example, 3-pentanol induced the SA and JA pathways against *Xanthomonas axonopodis* pv *vesicatoria*[11] and *P. syringae* pv. *tomato*.[20] Resistance induced by the volatile hexadecane was dependent on SA but not on JA.[15] Acetoin treatment invoked the SA, JA, and ethylene signaling pathways.[16]

In conclusion, we suggest that BVCs may more related ISR as plant protection mechanism of action. Pavlica and coauthors[21] declared that only a small number of soil volatiles such as formaldehyde and ammonia could reach a threshold concentration to reduce conidia germination of pathogenic fungi. There is a report that BVCs emission could be 30–200 ng/g depending on the soil type. However, bacteria are able to produce more than 30 g/L acetoin[22] and 2,3-butanediol[23] in synthetic media, while only 2–200 ng of these compound can be adequate to activate effective systemic resistance against *Erwinia carotovora*.[24] Up to 90% of conidia of *Cochliobolus victoria* germinated when they were exposed to BVCs in a soil sample in an open vial system.[25] In our previous work,[8] the BVCs acetoin, 2,3-butanediol, 3-pentanol, 1-pentanol, 2-hydroxy-3-pentanone, methyl jasmonate, and methyl SA did not affect the growth and spore formation of *B. cinerea* at a concentration of 100 μM, while this concentration significantly suppressed disease. Eventually, volatiles produced by bacteria normally act as infochemicals to communicate to other organisms in their niche and they can be toxic in specific conditions in which they are produced at high concentrations.

Abbreviations

BVC	bacterial volatile compound
ISR	induced systemic resistance
JA	jasmonic acid
SA	salicylic acid.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

We are grateful for financial support from the Industrial Source Technology Development Program of the Ministry of Knowledge Economy (10044909), the Next-Generation BioGreen 21 Program (SSAC grant #PJ01111803), RDA, and the KRIBB initiative program of South Korea.

References

[1] Schulz S, Dickschat JS. Bacterial volatiles: the smell of small organisms. Natl Prod Rep 2007; 24:814-42; PMID:17653361; http://dx.doi.org/10.1039/b507392h

[2] Kai M. Bacterial volatiles and their action potential. Appl Microbiol Biotechnol 2009; 81:1001-12; PMID:19020812; http://dx.doi.org/10.1007/s00253-008-1760-3

[3] Chen H, Xiao X, Wang J, Wu L, Zheng Z, Yu Z. Antagonistic effects of volatiles generated by *Bacillus subtilis* on spore germination and hyphal growth of the plant pathogen, *Botrytis cinerea*. Biotechnol Lett 2008; 30:919-23; PMID:18165869; http://dx.doi.org/10.1007/s10529-007-9626-9

[4] Li Q, Ning P, Zheng L, Huang J, Li G, Hsiang T. Fumigant activity of volatiles of *Streptomyces globisporus* JK-1 against *Penicillium italicum* on *Citrus microcarpa*. Postharvest Biol Technol 2010; 58:157-65; http://dx.doi.org/10.1016/j.postharvbio.2010.06.003

[5] Li Q, Ning P, Zheng L, Huang J, Li G, Hsiang T. Effects of volatile substances of *Streptomyces globisporus* JK-1 on control of *Botrytis cinerea* on tomato fruit. Biol Control 2012; 61:113-20; http://dx.doi.org/10.1016/j.biocontrol.2011.10.014

[6] Liu W-w, Mu W, Zhu B-y, Du Y-c, Liu F. Antagonistic activities of volatiles from four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens. Agr Sci China. 2008; 7(9):1104-14; http://dx.doi.org/10.1016/S1671-2927(08)60153-4

[7] Sharifi R, Ryu C-M. Are bacterial volatile compounds poisonous odors to a fungal pathogen *Botrytis cinerea*, alarm signals to Arabidopsis seedlings for eliciting induced resistance, or both? Front Microbiol 2016; 7:196; PMID:26941721; http://dx.doi.org/10.3389/fmicb.2016.00196

[8] Sharifi R, Ahmadzade M, Behboudi K, Ryu C- M. Role of *Bacillus subtilis* volatiles in induction of systemic resistance in Arabidopsis. Iranian J Plant Prot Sci 2013; 44:91-101

[9] Vespermann A, Kai M, Piechulla B. Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. Appl Environ Microbiol 2007; 73:5639-41; PMID:17601806; http://dx.doi.org/10.1128/AEM.01078-07

[10] Quintana-Rodriguez E, Morales-Vargas AT, Molina-Torres J, Ádame-Alvarez RM, Acosta-Gallegos JA, Heil M. Plant volatiles cause direct, induced and associational resistance in common bean to the fungal pathogen *Colletotrichum lindemuthianum*. J Ecol 2015; 103:250-60; http://dx.doi.org/10.1111/1365-2745.12340

[11] Choi HK, Song GC, Yi H-S, Ryu C-M. Field evaluation of the bacterial volatile derivative 3-pentanol in priming for induced resistance in pepper. J Chem Ecol 2014; 40:882-92; PMID:25149655; http://dx.doi.org/10.1007/s10886-014-0488-z

[12] Cortes-Barco A, Hsiang T, Goodwin P. Induced systemic resistance against three foliar diseases of *Agrostis stolonifera* by (2R,3R)-butanediol or an isoparaffin mixture. Ann Appl Biol 2010; 157:179-89; http://dx.doi.org/10.1111/j.1744-7348.2010.00417.x

[13] Kishimoto K, Matsui K, Ozawa R, Takabayashi J. Volatile 1-octen-3-ol induces a defensive response in *Arabidopsis thaliana*. J Gen Plant Pathol 2007; 73:35-7

[14] Lee B, Farag MA, Park HB, Kloepper JW, Lee SH, Ryu CM. Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*. PLoS One 2012; 7(11): e48744; PMID:23209558; http://dx.doi.org/10.1371/journal.pone.0048744

[15] Park HB, Lee B, Kloepper JW, Ryu C-M. One shot-two pathogens blocked: Exposure of Arabidopsis to hexadecane, a long chain volatile organic compound, confers induced resistance against both *Pectobacterium carotovorum* and *Pseudomonas syringae*. Plant Signal Behav 2013; 8:e24619; PMID:23603940; http://dx.doi.org/10.4161/psb.24619

[16] Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Pare PW, Bais H.P. The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Commun Integr Biol 2010; 3:130-8; PMID:20585504; http://dx.doi.org/10.4161/cib.3.2.10584

[17] Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH, Cho BH, Yang KY, Ryu CM, Kim YC. 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. Mol Plant Microbe Interact 2008; 21:1067-75; PMID:18616403; http://dx.doi.org/10.1094/MPMI-21-8-1067

[18] Blom D, Fabbri C, Eberl L, Weisskopf L. Volatile-mediated killing of *Arabidopsis thaliana* by bacteria is mainly due to hydrogen cyanide. Appl Environ Microbiol 2011; 77:1000-8

[19] Effmert U, Kalderás J, Warnke R, Piechulla B. Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 2012; 38: 665-703; PMID:22653567; http://dx.doi.org/10.1007/s10886-012-0135-5

[20] Song GC, Choi HK, Ryu C-M. Gaseous 3-pentanol primes plant immunity against a bacterial speck pathogen, *Pseudomonas syringae* pv. tomato via salicylic acid and jasmonic acid-dependent signaling pathways in Arabidopsis. Front Plant Sci 2015; 6:821; PMID:26500665; http://dx.doi.org/10.3389/fpls.2015.00821

[21] Pavlica DA, Hora TS, Bradshaw JJ, Skogerboe RK, Baker R. Volatiles from soil influencing activities of soil fungi. Phytopathology 1978; 68, 758-65; http://dx.doi.org/10.1094/Phyto-68-758

[22] Xiao ZJ, Liu PH, Qin JY, Xu P. Statistical optimization of medium components for enhanced acetoin production from molasses and soybean meal hydrolysate. Appl Microbiol Biotechnol 2007; 74:61-8; PMID:17043817; http://dx.doi.org/10.1007/s00253-006-0646-5

[23] Ma C, Wang A, Qin J, Li L, Ai X, Jiang T, Tang H, Xu P. Enhanced 2,3-butanediol production by *Klebsiella pneumoniae* SDM. Appl Microbiol Biotechnol 2009; 82:49-57; PMID:18949476; http://dx.doi.org/10.1007/s00253-008-1732-7

[24] Ryu C-M, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW. Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 2004; 134:1017-26; PMID:14976231; http://dx.doi.org/10.1104/pp.103.026583

[25] Liebman JA, Epstein L. Partial characterization of volatile fungistatic compound(s) from soil. Phytopathology 1994; 84:442-6; http://dx.doi.org/10.1094/Phyto-84-442