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

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ABSTRACT

Bacterial volatiles protect plants either by directly inhibiting a pathogen 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.

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Bacteria emit a vast array of volatile organic compounds belonging to various chemical groups. More than 120 individual volatiles have been identified in actinomycetes, a group of Gram-positive bacteria. 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, induce resistance to biotic and abiotic stresses, and promote or suppress plant growth.

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 and B. cinerea. In our previous work, we revealed that the effect of volatiles on B. cinerea was dose-dependent. 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. Quintana-Rodriguez and coworkers 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. 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 spor germination of the mycorrhizal fungus Glomus mosseae. Acetoin, 2,3-butanediol, and 3-pentanol improved fungal growth and spore production of B. cinerea at a concentration of 100 μM.

Secondly, volatiles can protect plants via induced systemic resistance (ISR) against pathogens. The long-chain volatiles tridecane and hexadecane 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%. The same concentration of acetoin induced resistance against P. syringae pv. tomato. Acetoin resistance in Arabidopsis. In our previous work, volatiles of B. subtilis GB03 and 100 μM 2-hydroxy-3-pentanone suppressed the growth of B. cinerea on Arabidopsis.

In our previous work, we designed an experiment to determine the contribution of each mechanism, direct fungial inhibition or boosting of plant immunity, to protection of Arabidopsis against B. cinerea. We found that a low
concentration of volatiles was sufficient to elicit induced resistance in plants, but was not sufficient to inhibit fungal growth and development. ISR and direct fungal inhibition were responsible for more than 90% and less than 10% of plant protection, respectively (Fig. 1). 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.

Volatile of *B. subtilis* GB03 primed the expression of PRI and PDF1.2, 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* and *P. syringae pv. tomato.* Resistance induced by the volatile hexadecane was dependent on SA but not on JA. Acetoin treatment invoked the SA, JA, and ethylene signaling pathways.

In conclusion, we suggest that BVCs may more related ISR as plant protection mechanism of action. Pavlica and coauthors 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 and 2,3-butanediol in synthetic media, while only 2–200 ng of these compound can be adequate to activate effective systemic resistance against *Erwinia carotovora.* Up to 90% of conidia of *Cochliobolus victoria* germinated when they were exposed to BVCs in a soil sample in an open vial system. In our previous work, 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.

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