Dynamic communication between plants and rhizobacteria via volatile signals

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Abstract

Certain plant growth-promoting rhizobacteria (PGPR), in the absence of physical contact with plants, stimulate plant growth and elicit induced systemic resistance (ISR) via volatile organic compound (VOC) emissions. Gas chromatographic analysis of VOCs collected from the PGPR strains Bacillus subtilis strain GB03 and B. amyloliquefaciens strain IN937a reveals consistent patterns in VOC emissions in comparison with non-growth promoting strains such as E. coli DH5α. The two most abundant compounds, 2,3-butanediol and 3-hydroxy-2-butanone, are consistently emitted from GB03 and IN937a while these metabolites are not released from DH5α. Transcriptional approaches have been employed to prove how Arabidopsis respond to biologically active bacterial VOCs. To assess potential utilization of PGPR VOCs for crop plants, volatile blends from GB03, IN937a, and DH5a have been applied separately to pepper, tomato, and cucumber roots. Bacterial survival capacity of 2,3-butanediol null mutants was significantly reduced in proximity with plant tissue. These reduced bacterial survival rates suggest that in addition to bacterial VOCs triggering plant growth and induced systemic resistance in plants, such chemicals provide protection for

PGPR via chemical signaling within the host plant.

Rhizobacteria help plant growth and defense against multiple pathogens

Plant growth-promoting rhizobacteria (PGPR) include a wide range of root-colonizing bacteria with the capacity to enhance plant growth by increasing seed emergence, plant weight, and crop yields (Kloepper, 1980). Seed or seedling treatments with PGPR have been used to enhance growth of several crops (Glick, 1995) as well as to suppress the growth of plant pathogens and deleterious rhizosphere microorganisms. Proposed mechanisms for plant growth promotion by PGPR include bacterial synthesis of the plant hormones indole-3-acetic acid (IAA), cytokinin, and gibberellin; breakdown of plant-produced ethylene by bacterial production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase; and increased mineral and nitrogen availability (Glick et al., 1995; 1999).

In addition to promoting plant growth, application of certain PGPR strains to seeds or seedlings leads to a state of induced systemic resistance (ISR) in the treated plant (Kloepper et al., 1992). PGPR can influence pathogens by direct or indirect effects. Indirectly, root-colonization by certain rhizobacteria induces systemic resistance that is effective against viral, bacterial, and fungal pathogens, as well as nematodes (Kloepper et al., 1999). ISR occurs in many plants including carnation, cucumber, tobacco, tomato, bean, radish, and *Arabidopsis* (Van Loon et al., 1998; Ryu et al., 2003b; 2004b). In contrast to signaling pathways of necrotic pathogens or chemical-elicited systemic acquired resistance, ISR does not require a salicylic acid-dependent pathway (Van Loon et al., 1998; Ryu et al., 2003b; 2004b). Several microbial determinants have been associated with elicitation of ISR: 2,4-diacetylphloroglucinol (Iavicoli et al., 2003), the O-antigen from lipopolysaccharide (Van Peer and Schippers, 1992), and salicylic acid (Maurhofer et al., 1998).

Bacterial volatiles are as aromatic stimulants for plant growth and defense

By physically separating plant growth promotion rhizoobacteria from their host plant airborne chemicals from certain soil bacteria have been identified as effective signals for triggering plant growth and ISR. (Ryu *et al*, 2003a; Ryu *et al*, 2004a). Of several PGPR tested, two strains, *Bacilus subtilis* GB03 and *B. amyloliquefaciens* IN937a, elicited plant growth promotion and ISR

by volatile organic compounds (VOCs) emissions. In the case of growth promotion by VOCs from strain GB03, the cytokinin receptor-deficient (*cre*1) and cytokinin- and ethylene-insensitive (*ein*2) mutants were insensitive to growth promotion effects triggered by GB03 volatiles, while ethylene-insensitive (*etr*1), auxin-transporter-deficient and ethylene-insensitive (*eir*1), gibberellic acid-insensitive (*gai*2), and brassinosteroid-insensitive (*cbb*1) mutants were all sensitive to GB03 VOCs as evidenced by measurement of growth promotion.

Of several Arabidopsis mutant lines tested for regulatory control of ISR against *Erwinia carotovora* subsp. *carotovora*, only the ethylene-insensitive line (*ein*2) did not exhibit an amelioration of disease symptoms when Arabidopsis plants were pre-treated with GB03 volatiles. To examine if ISR is mediated at the level of transcription, three transgenic β-glucuronidase (GUS) fusion lines involved in plant-defense signaling were tested. Of those lines assayed, the jasmonic acid and ethylene-responsive PDF 1.2 gene GUS fusion plant alone exhibited elevated GUS activity (>1000 fold) over untreated control plants (Ryu *et al*, 2004).

Besides study of strain GB03 VOCs, characterization of the VOCs produced by IN937a, coupled with bioassays of fractions of VOCs elicited plant growth promotion allowed for an initial characterization of potentially active bacterial volatiles. Exposure of *Arabidopsis* to VOCs from strain IN937a resulted in a significant reduction in disease severity caused by *E. carotovora* subsp. *carotovora*. Elicitation of ISR occurred with exposure to bacterial VOCs for as little as 4 days. Various mutant lines of *Arabidopsis* were exposed to whole VOCs. Mutant lines included a jasmonic acid-insensitive line (*coi1*), an ethylene-insensitive line (*ein2*), a salicylic acid-degrading line (NahG), and a line that lacks a regulatory gene (*npr1*). VOCs from strain IN937a elicited ISR on all of these lines. Hence, elicitation of ISR by VOCs of IN937a is independent of jasmonic acid, ethylene, salicylic acid, and *npr1*. Such a pattern of signal pathway has not been reported with ISR elicited by bacteria, and therefore, it is likely that VOCs of IN937a elicit a distinct and uncharacterized pathway in *Arabidopsis.* The two most abundant compounds, 2,3-butanediol and 3-hydroxy-2-butanone (also referred to as acetoin), were consistently released from strainsGB03 and IN937a while these metabolites were not released from DH5α or water-treated MS media. Other components of the complex bacterial bouquet that did not elicit ISR included dodecane, 2-undecanone, 2-tridecanone, 2-tridecanol, and tetramethyl pyrazine.

In *Bacillus* sp., 2,3-butanediol and acetoin are produced with low atmospheric O_2 partial pressure to provide an alternative electron sink for the

regeneration of NAD^+ when aerobic respiration is limited possible. This additional metabolic pathway functions analogously to alcohol fermentation activated in yeast under anaerobic conditions. The biological activity of 2,3 butanediol in triggering ISR was surmised in *Arabidopsis* when pre-exposure of plants to low doses (pg to ng range) of 2,3-butanediol activated ISR. The priming activity of 2,3-butanediol to reduce a plant's susceptibility to disease was confirmed when Bacilli strains genetically blocked in the production of 2,3-butanediol exhibited no disease protection. More interestingly, direct application of bacterial volatiles into plant roots increased plant growth significantly and elicited ISR in the greenhouse (unpublished data).

Uncharacterized bacterial odors

The involvement of known signaling pathways in Arabidopsis were screened by exposing defined mutants and transgenic plant lines to bacterial emissions containing 2,3-butanediol. ISR triggered by strain GB03 VOC emissions was independent of salicylic acid, NPR1, and jasmonic acid signaling pathways, but did appear to be mediated by ethylene. Interestingly plant growth promotion activation by strain IN937a was independent of all the signaling pathways that were tested suggesting that additional VOCs utilize alternative pathways to trigger plant growth promotion (Ryu et al., 2003a). In fact, solid phase microextraction (SPME) analysis of bacterial odors turned up substantial differences in VOC profiles between two active strains GB03 and IN937a for growth promotion. Major branched-chain alcohols 3-methyl-1-butanol, 2-methyl-1-butanol and their conjugates were detected in IN937a, albeit not in GB03. Differences in volatile profiles might account for the dissimilarity observed among both strains in triggering growth promotion via volatile chemical signals, and suggests the existence of diverse VOC metabolism existing in two closely related Bacilli species (Farag et al., 2006).

Plant and bacteria responses to rhizobacterial volatiles

To understand plant-signaling pathways induced by bacterial VOCs during plant growth promotion and ISR, we have begun to characterize global changes in the *Arabidopsis* transcriptome using available microarray technology. Microarray results revealed physiological changes associated with growth, photosynthesis, and stress tolerance. In addition to bacterial synthesis of plant growth regulators such as auxin, bacterial VOCs induce auxin synthesis and transport *in planta*. Transcriptional analysis also

identified changes in cell wall loosening providing a possible mechanism for rapid cell expansion and leaf growth promotion that is observed in GB03 VOC exposure (Zhang et al 2007). 2,3-Butanediol also appears to participate in bacterial colization with its host.. In case of *Vibrio cholerae* the mutant defective in 2,3-butanediol synthesis exhibited reduced capacity to colonize the gut of its host the mouse and did not develop its usual biofilm. Preliminary observations with the 2,3-butanediol non-producing *Bacillus subtilis* strain also indicates poor colonization of its host, in this case, pepper roots and overall poor bacterial fitness *in situ*. It appears that 2,3-butanediol produced by *B. subtilis* may serve in a dual functions eliciting the production of plant antimicrobial compounds and as a protecting agent for bacterial cells (Yi et al., unpublished data). Additional investigations are underway to better characterize how PGPR utilize volatile signaling to transmit information between plants and rhizobacteria.

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