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Article Addendum

Arabidopsis thaliana Root Surface Chemistry Regulates in Planta Biofilm Formation of *Bacillus subtilis*

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PGPR, biofilm, catechol and ROS

Addendum to:

A Degradation Product of the Salicylic Acid Pathway Triggers Oxidative Stress Resulting in Downregulation of Bacillus subtilis *Biofilm Formation on* Arabidopsis thaliana *roots.*

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ABSTRACT

Among the various rhizospheric interactions, plant root-microbe interactions are very important both economically and ecologically. The interaction of plant roots with plant growth promoting rhizobacteria (PGPR) have been studied in case of symbiotic organisms. However, the knowledge on interaction with other PGPRs such as biocontrol *Bacillus* sps. is vastly unexplored. Especially the complex root surface chemistry and its effect on modulating the bacterial growth and association with the root system has not been investigated. Recently, by adopting a systematic stepwise experimental approach we unraveled the importance of root plane chemistry on the colonization and biofilm formation by *B. subtilis*, an important biocontrol-PGPR. This study may further increase our understanding in the field of rhizosphere biology and area of root secretions and their possible role in plant microbe interactions.

Rhizosphere, the region around the roots harbor wide array of microbial populations, which may be beneficial, neutral or detrimental to plant growth. The reason for this efficient colonization and the presence of increased microbial populations has been ascribed to the nutrient rich environment of the rhizosphere. It has been reported that nearly 40% of total plant photosynthates are secreted through root exudates.[1] The studies appreciating the role of root exudates in rhizospheric interactions have been beginning to appear. Among the different groups of microbes which colonize the rhizosphere and the root surface, the plant growth promoting rhizobacteria (PGPR) are a class, which promotes plant growth.[2] The plant growth promotion by such PGPRs is primarily rendered by their ability to produce phytohormones, improve the nutrient uptake and protection from pathogenic microorganisms.[3] The mechanism by which the biocontrol-PGPR, *Bacillus subtilis* protects plant roots from pathogenic bacteria include biofilm formation in addition to antibiotic and surfactin production.[4,5] Biofilms are structured community of microbial cells encased in a self-produced polymeric matrix.[6] Recent research studies have begun to understand and elucidate the genetic pathways controlling the *B. subtilis* biofilm formation.[7-15] No previous study has attempted the question whether, a PGPR, *B. subtilis* is recognized like pathogen through the well-studied pathogenesis/disease resistance pathways? We employed various plant disease resistance pathway mutants and a transgenic line to determine whether *A. thaliana* can distinguish between a PGPR, such as *B. subtilis*, and a plant pathogenic bacterium. In contrast to our initial speculation, this screening step showed that *B. subtilis* is not recognized as a pathogen by *A. thaliana* roots as it could colonize the roots of all the plant disease pathway mutants studied. This result though not fully conclusive indicated that *A. thaliana* roots interact with the *B. subtilis* through a pathway other than the regular pathogenesis/disease resistance pathways. This forms an important clue for the future studies involving the *A. thaliana-B. subtilis* interactions. However, we observed the non-colonization and suppression of *B. subtilis* biofilm formation on the roots of *A. thaliana* line *NahG* (Fig. 1), a transgenic line-containing gene for salicylate hydroxylase, which hydrolyzes salicylic acid and results in the overproduction of catechol.[16] This suggested that the catechol might be playing a key role in inhibiting the *B. subtilis* colonization and biofilm formation on the *NahG* root surface. This speculation was further tested by studying the effect of catechol on in vitro biofilm formation on the abiotic surface and in vivo on the wild type Col-0 plants which showed the suppression of biofilm formation under both the conditions. There is a published evidence that showed that *A. thaliana* non-host resistance is compromised in *NahG* plants in response to *Pseudomonas syringae* pv. *phaseolicola* 3121.[16] However our results showed that the catechol acts on *B. subtilis* through a pathway, which is altogether different from the mechanism reported for a pathogenic interaction.[16]

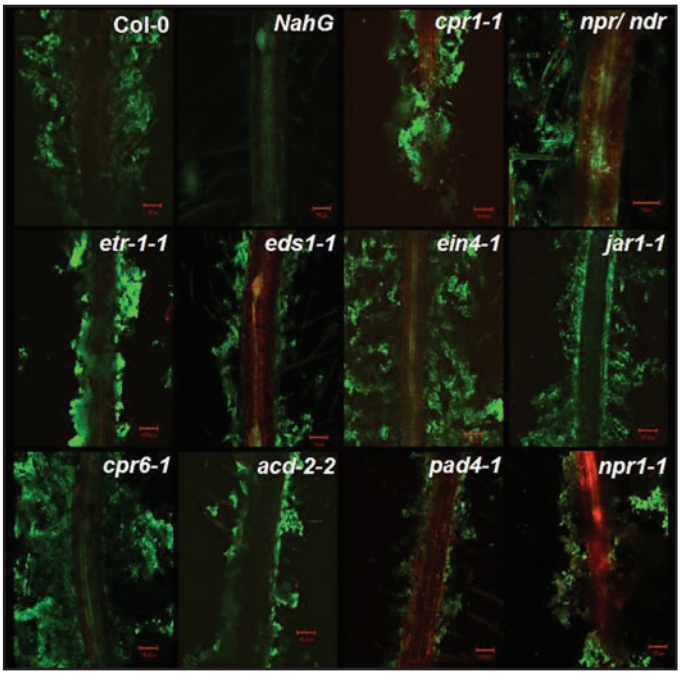

Figure 1. Confocal microscopy images of Arabidopsis roots co-cultivated with *B. subtilis* strain FB-17 showing dense biofilm formation on the surface of Col-0, *cpr6-1, etr1-1, jar1-1, ein4-1,* less biofilm formation on *cpr1-1 pad4-1, eds1-1, npr1-1, npr/ndr* double mutant compared to the *NahG* roots.

The lack of biofilm formation on the *NahG* root surface and a threefold reduction in the *B. subtilis* cell surface adhesion led us to further speculate that the additional inhibition might occur directly on the root surface through a specific catechol induced biochemical changes. Catechol is a phenolic compound and such compounds are known to generate reactive oxygen species (ROS),[17] we hypothesized that the inhibition might be brought about by the higher titers of ROS generated by increased concentrations of catechol on *NahG* roots. In accordance with our hypothesis the *NahG* root surface stained and imaged for ROS showed higher ROS generation when compared to all other mutants and wild type Col-0.[18] Similarly, a significantly higher titres of surface and exuded ROS production was observed in *NahG* when compared to Col-0. However, the role of ROS was further conclusively established when the biofilm formation was restored in the *NahG* roots treated with ascorbic acid, a ROS quencher. Further, we hypothesized that the suppressed binding of *B. subtilis* due to altered root surface chemistry, might be occurring through the direct suppression of transcriptional operons required for biofilm formation in *B. subtilis*. In consistent with all the previous results direct catechol treatment resulted in a significant reduction in the transcription levels of the operons *yqxM* and *epsA* which are required for biofilm formation in *B. subtilis*. Finally, with these transcriptional profiling studies, we showed that the suppression of *B. subtilis* biofilm formation on *NahG* root surface is due to the presence of catechol on the *NahG* root surface (and in the surrounding area), resulting in ROS mediated down regulation of genes required for biofilm formation in *Bacillus subtilis*.

Our findings established the importance of root surface chemistry and secretions in colonization and biofilm formation of *B. subtilis*. There is a possibility that the biocontrol mechanism driven by biofilm formation is regulated by in planta redox potential in the rhizosphere. It is clear, however, that additional information is required to further clarify the role of gene expression in biofilm formation and efficacy. Studies in rice and Arabidopsis systems have demonstrated that higher catechol levels result in the production of superoxides and H_2O_2 leading to increased ROS generation.[16,19] Further mechanistic studies are required to elucidate the role of catechol and catechol generated ROS in the rhizospheric interactions such as plant-plant and plant-microbe and microbe-microbe interactions. In addition, research studies focusing on the elucidation of novel plant genes that are detrimental in *B. subtilis* colonization on *A. thaliana* will shed some more light on this beneficial interaction.

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