In wild tobacco, *Nicotiana attenuata*, variation among bacterial communities of isogenic plants is mainly shaped by the local soil microbiota independently of the plants’ capacity to produce jasmonic acid

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The phytohormone jasmonic acid (JA) plays a central role in defense against necrotrophic pathogens and herbivores in *Nicotiana attenuata*. Recently Santhanam et al. showed that JA does not have a major role in shaping the root- and shoot associated bacterial communities, though a few taxa differed among control (empty vector, EV) plants and plants impaired in their capacity to produce JA (irAOC). In this addendum, we provide additional data showing that the composition of the plant bacterial communities is mainly shaped by tissue type. The qualitative data analysis revealed that at the order level, 5 bacterial OTUs formed a core community found in all tissues irrespective of genotypes, while 9 OTUs were different among roots and shoots. The heterogeneity among individual plants was high masking the potential genotype effect on bacterial communities. Using a culture-dependent approach, 3 of 18 bacterial taxa retrieved either only from one of the genotypes or from both had a growth promoting effect on EV and irAOC seedlings. The data suggest that the local soil niche in which the roots grows is a major driver of the variability in root bacterial communities recruited by different individuals, and the plant growth-promoting effects of some taxa are independent of the genotype.

Plants harbor a diverse range of bacterial communities which are influenced by many biotic and abiotic factors. Several studies showed that tissue types such as leaves and roots influence the bacterial community composition, and harbor distinct communities. It is often assumed that root bacterial communities are shaped by soil microbiota, and leaf bacterial communities by air, sunlight irradiation, stomata and mineral content of the leaves. However, 4 independent studies using *Arabidopsis* as a model system indicated that at the phylum level, core communities such as Actinobacteria, Bacteriodetes and Proteobacteria can be found in all roots independent of soil type and genotypes, strongly indicating that bacteria do not randomly colonize roots, but certain phyla preferentially colonize plant roots.

In a previous study, we analyzed leaf and root bacterial community of isogenic field grown plants impaired in JA-production (irAOC) and control plants (empty vector, EV) by culture dependent and independent (pyrosequencing) approaches. Based on the quantitative data, we showed that leaf bacterial communities are different from those of roots. Here, we demonstrate that based on qualitative data (presence and absence of OTUs at 97% similarity) leaf bacterial communities are clearly distinct from roots (Fig. 1A), and within each tissue type (root vs leaf) plants impaired in JA production and EV plants do not show a genotype-specific pattern. These data are consistent with our previous findings, and similar results were obtained by Bodenhausen et al., who showed that leaf bacterial communities of *Arabidopsis thaliana* are different from those of roots and concluded that organ type (root vs leaf) type influences the composition of the bacterial communities.

Though root and leaf bacterial communities were clearly distinct, a bacterial core community was present in all
samples of field-grown *N. attenuata* plants irrespective of the genotype (Fig. 1B). At the order level, this core community consisted of 5 OTUs retrieved from all roots and shoots from 27 OTUs found in total. These 5 OTUs belonged to the bacterial phyla Bacteriodetes, Proteobacteria and Firmicutes. Additionally, 9 OTUs were present in all root samples, representing also 2 additional bacterial phyla, Actinobacteria and Deinococcus-thermus, indicating a root-specific enrichment. In accordance with our study, Actinobacteria were shown in a previous studies to be enriched in roots irrespective of soil types.\(^5,^6\) Deinococcus-thermus taxa are described as highly resistant to environmental hazards and can survive high doses of gamma and UV radiation.\(^11,^12\) Interestingly, *N. attenuata*’s native habitat, the Great Basin Desert, Utah, USA\(^13\) is characterized by high light intensities and high UV-B fluence rates. In earlier studies, independent of the soil type, the bacterial community composition was found to be similar at the phylum level in different genotypes of the same and related species. Only few bacterial taxa were quantitatively different among plant genotypes\(^1,^5-^7\) suggesting that genotypes have a minor role in structuring bacterial communities. Based on 16S rDNA gene pyrosequencing of 8 *Arabidopsis* ecotypes roots Lundberg et al\(^5\) showed that of 778 OTUs, only 12 OTUs exhibited host genotype specific quantitative enrichment. In another study, using the same technique, Bulgarelli et al\(^6\) found only one OTU was significantly different among 2 *Arabidopsis* ecotypes. In our study, based on ANOSIM  β diversity, the overall bacterial diversity of leaves and roots of EV and irAOC genotypes was not significantly different,\(^1\) and we did not find a consistent clustering based on genotype (Fig. 1A) by qualitative data, and the bacterial community composition was highly heterogeneous among replicate plants based on qualitative and quantitative data. At the genera level, 21 OTUs significantly differed among EV and irAOC genotypes,\(^1\) and at the order level, 1 OTU was distinct among the genotypes (Fig. 1B). We hypothesize that sample-to-sample differences are due to differences in the local soil bacterial community in which the plant grows (Fig. 2), leading to the recruitment of soil-specific taxa. This hypothesis is in line with a large-scale study using 27 maize genotypes growing at 5 different locations in the US. Bacterial communities clearly clustered by soil, but not by genotype.\(^14\)
Bacterial communities, which reside in plants can either have beneficial or detrimental effects on their hosts.\textsuperscript{15,16} Detrimental effects are caused by necrotrophic and biotrophic pathogens \textsuperscript{17,18} Beneficial effects can be direct or indirect, resulting in plant growth promotion. Direct plant growth promotion (PGP) can result from improved nutrient acquisition (e.g. nitrogen, phosphorous), the production of phytohormones (IAA, gibberellins) or the synthesis of stress modulators such as 1-amino cyclopropane-1-carboxylate (ACC) deaminase which lowers the plant’s endogenous ethylene levels.\textsuperscript{3,19} Indirect plant growth promotion can be due to the prevention or reduction of pathogen infection either by direct suppression, e.g., outcompeting pathogens for nutrients or by priming tissues for enhanced defense against pathogen or herbivore attack.\textsuperscript{20,21} The enrichment of certain beneficial bacterial taxa can lead to opportunistic mutualisms between plants and microbes, as exemplified by a recent study of Meldau et al\textsuperscript{22,23} showing that an ethylene insensitive \textit{N. attenuata} genotype can recruit beneficial microbes to compensate its growth deficiency. PGP traits of certain bacterial isolates can be host dependent; e.g. Long et al\textsuperscript{24} showed that bacterial strains isolated from \textit{Solanum nigrum} roots were unable to promote growth of \textit{N. attenuata}. However, in general, large numbers of bacterial isolates enhance growth of plants independent of their hosts.\textsuperscript{3,19,20} The exact mechanism responsible for the recruitment and fine tuning of bacterial taxa from the local soil community remains to be elucidated. Some studies indicate that the lignin content and cell wall composition may play a role,\textsuperscript{2,25} in addition to ethylene signaling.\textsuperscript{26}

In our study, we isolated 414 bacterial strains from surface sterilized roots and leaves of both genotypes using a culture-dependent approach.\textsuperscript{1} 18 strains were classified as putative specialists and generalists based on the isolation of a particular strain from either of the 2 plant genotypes (EV or irAOC). PGP effects of these putative generalist and specialist bacterial strains were investigated (for experimental details see “In-vitro re-isolation” in Santhanam et al\textsuperscript{1}). Three of the 18 strains (\textit{B. cereus} CN2, \textit{P. azotoformans} A70 \textit{A. nitroguajacolicus} E46, \(n = 6\), \(P < 0.05\), Fisher’s PLSD) used in this assay significantly promoted plant growth with respect to plant biomass (ANOVA; \(F_{19,90} = 6.81\), \(P < 0.001\)), leaf surface area (ANOVA; \(F_{19,90} = 3.58\), \(P < 0.001\)) and primary root length (ANOVA; \(F_{19,90} = 19.84\), \(P < 0.001\), Fig. 3). Four isolates significantly reduced plant growth (\(n = 6\), \(P < 0.05\), Fisher’s PLSD, Fig. 3), while 11 isolates had no significant effect (Fig. 3). The PGP effects did not depend on the plant’s genotype from which they were
isolated (2-way ANOVA plant biomass- bacterial type*genotype: $p = 0.98$, leaf surface area- bacterial type*genotype: $p = 0.99$, root length- bacterial type*genotype: $p = 0.97$). Interestingly, inoculation with a mixture of all 18 bacterial isolates also did not promote growth, though - based on the single inoculations - plant growth promoting bacteria were present. We assume that under in-vitro conditions growth promotion by these bacterial isolates might be inhibited by competitive interactions among different isolates in mixed inoculations. In order to test whether colonization of bacterial isolates correlate with the PGP effects,
we performed a linear regression model with both genotypes; however, no correlation was observed between PGP effects and the colonization pattern (EV- plant biomass: $R^2 = 0.07$, $p = 0.274$, leaf surface area: $R^2 = 0.05$, $p = 0.337$, root length: $R^2 = 0.05$, $p = 0.336$; irAOC- plant biomass: $R^2 = 0.074$, $p = 0.258$, leaf surface area: $R^2 = 0.04$, $p = 0.39$, root length: $R^2 = 0.05$, $p = 0.319$). This analysis corroborates that PGP effects were independent of quantitative root colonization and genotype and might be influenced by bacterial PGP traits such as production of IAA and ACC deaminase activity as exemplified by Long et al. 

Based on these results, we conclude that leaves and roots of field grown N. attenuata plants harbor distinct bacterial communities. Though all field-grown plants show a core community of the bacterial root microbiota in Arabidopsis thaliana and may increase the plant’s fitness depending on the environmental conditions.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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