The banana microbiome: stability and potential health indicators

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Abstract

Banana cultivation represents one of the world's largest monocultures, and *Musa* spp. belong to the most important global food commodities. Although the plant-associated microbiome has substantial influence on plant growth and health, there is limited knowledge of the banana microbiome and its influencing factors. We studied the impact of i) biogeography, ii) agroforestry, and iii) Fusarium wilt (race 1) infestation on the banana-associated gammaproteobacterial microbiome analyzing 'Gros Michel' (AAA genome) grown in Nicaragua and Costa Rica. Additionally, we investigated iv) the microbiome of the East African highland bananas (EAHB, AAA genome) treated with different organic soil amendment combinations of mulch and manure, and v) the microbiome stability of Xanthomonas wilt-resistant transgenic 'Sukari Ndizi' (AAB genome) in Uganda. Overall, the gammaproteobacterial banana microbiome was dominated by *Pseudomonadales*, *Enterobacteriales* and *Xanthomonadales*. An extraordinary high diversity of gammaproteobacterial microbiota was observed within the endophytic microenvironments, endorhiza and pseudostem. Enterobacteria were identified as a highly dominant group in aerial plant parts, especially of the pseudostem and leaves. Our studies show that the gammaproteobacterial banana microbiome is influenced by biogeography and banana cultivar. Work also corroborates findings that agroforestry leads to increased plant health via shifts within the microbiome. In this light, a sophisticated design of manipulating plant microbiomes can help us reduce pesticide and fertilizer use. Using gammaproteobacterial health indicators can help preventing Fusarium wilt infestation. Organic management practices lead to increased microbial soil diversity. Further studies based on more holistic population microbiology are necessary to potentially extend this behavior to the entire microbial community. Our risk assessment shows a stable microbiome for Xanthomonas wilt-resistant transgenic bananas with no consequences for non-target rhizobacteria, although additional studies should address the microbiome stability over a longer time frame.

Keywords: agroforestry, banana-associated microbiota, organic soil amendments, Fusarium wilt, Xanthomonas wilt

THE PLANT MICROBIOME AND THE SPECIAL ROLE OF GAMMAPROTEOBACTERIA

Plants, like other eukaryotes, form close interactions with microorganisms which are essential for the performance and survival of the host. Co-evolution has resulted in intimate plant-microbe relationships that create specific and stable microbiomes (Guttman et al., 2014). In addition to the joint fulfilment of tasks, some – even essential – functions are "outsourced" to symbiotic organisms living with them (Gilbert et al., 2012). Thus, in nature, plants and their inhabiting communities can collectively be considered as interacting meta-organisms or holobionts: an association of the macroscopic host and a diverse microbiome consisting of bacteria, archaea, fungi, and protists, within which the microbes usually outnumber the cells belonging to the plant host (Berg et al., 2016). In this respect, many studies in the last decades have shown that plant-microbe interactions are a key for

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understanding plant growth and health but also for sustainable crop production. Beneficial interactions of certain plant-associated – mainly rhizospheric – microorganisms with their host in plant growth, stress tolerance, and pathogen suppression are well investigated, and several microbe-based products have already been patented and are successfully applied in different agricultural production systems worldwide (Berg, 2009; Lugtenberg and Kamilova, 2009). Although still far from gaining dominance over chemical pesticide and fertilizer applications, the use of plant growth-promoting bacteria and biological control agents for the development of environmentally sound and sustainable agricultural management practices is constantly rising (Weller, 2007; Berg et al., 2013).

The structure of the plant microbiome is determined by biotic and abiotic factors, and it reveals high plant specificity, even at cultivar level (Berg and Smalla, 2009; Bulgarelli et al., 2012). The colonization of plants by microorganisms is a targeted process that is underlined by the existence of specific co-occurrence patterns and microbial networks (Cardinale et al., 2015). These networks are related to colonization resistance patterns, which determine the potential for allochthonous microorganisms (pathogens but also biological control agents) to invade the autochthonous community. Specific microorganisms are often vertically passed down from previous generations via the seeds (Hardoim et al., 2012) and likewise are horizontally transmitted between plants through pollen grains (Fürnkranz et al., 2012). In addition, structural diversity is paramount to the preventive avoidance of pathogen invasion/outbreaks (van Elsas et al., 2012).

Gammaproteobacteria are a dominant and important group of the overall plant microbiome (Fürnkranz et al., 2012; Rastogi et al., 2012) comprising a variety of plant growth-promoting (rhizo)bacteria and biological control agents. Probably the most prominent example in this context is the group of the fluorescent pseudomonads, which are well-known for their plant-beneficial properties (Weller, 2007) and the base of some commercially available products (Berg, 2009). Other examples for promising gammaproteobacterial plant growth promoters and pathogen suppressors are for instance the Xanthomonadales species *Stenotrophomonas rhizophila* (Alavi et al., 2013) or the Enterobacteriales species *Serratia plymuthica* (Adam et al., 2016). However, the group of Gammaproteobacteria also comprises some potential plant pathogenic members (Eastgate, 2000; Fürnkranz et al., 2012) and also encompasses species which were identified to be the causal agents of (opportunistic) infections in humans (Berg et al., 2015) and also of severe foodborne disease outbreaks (Buchholz et al., 2011; Bloch et al., 2012).

THE GAMMAPROTEOBACTERIAL BANANA MICROBIOME AND THE IMPACT OF BIOGEOGRAPHY AND BANANA CULTIVAR

In order to obtain an almost complete picture of the banana-colonizing Gammaproteobacteria under varying conditions, our studies targeted diverse plant parts and microenvironments: the rhizosphere soil surrounding the roots (which represents the interface with the bulk soil), the inner tissue of the roots – the endorhiza, the banana leaves and fruits, as well as the pseudostem (Rossmann et al., 2012; Köberl et al., 2015, 2017; Nimusiima et al., 2015). In comparison to other plants, an extraordinary high diversity of the gammaproteobacterial microbiota was observed within the endophytic microenvironments – endorhiza and pseudostem endosphere. The cylindrical succulent pseudostem is a peculiarity of the herbaceous banana plant which consists of closely packed leaf-petiole sheaths (Saravanan and Aradhya, 2011). It provides a unique microhabitat for endophytic microorganisms and was identified as a bacterial hot spot colonized by a high abundance and diversity of enterics and other Gammaproteobacteria (Rossmann et al., 2012; Köberl et al., 2015). The generally high diversity within the endophytic community of the banana plant can be explained by the permanent nature of its corm serving as a reservoir for endophytic diversity and the transmission to following generations via vegetative suckers.

Detailed colonization studies with focus on the class of Gammaproteobacteria revealed *Pseudomonadales*, *Enterobacteriales*, *Xanthomonadales* and *Legionellales* as the most dominant orders in the overall banana microbiome and linked preferences of specific taxa to individual plant parts, banana cultivars and banana-growing regions (Figure 1). The rhizosphere microbiome of 'Sukari Ndizi' (AAB genome) grown in Uganda was greatly dominated by *Xanthomonadales* (Nimusiima et al., 2015). This was in contrast to 'Gros Michel' (AAA genome) grown in Central America (Köberl et al., 2015, 2017) and also to East African highland bananas (EAHB, AAA genome) grown in Uganda (Rossmann et al., 2012), both revealing Pseudomonadales as the most dominant gammaproteobacterial order. 'Gros Michel' bananas – especially those from Costa Rica – and 'Sukari Ndizi' revealed a surprisingly high proportion of *Legionellales* in their rhizosphere communities, which were nearly completely absent in the investigated EAHBs. However, despite these disparities in the rhizosphere communities, the above-ground colonization revealed a relatively similar structure in all analyzed banana plants, almost exclusively inhabited by *Pseudomonadales* and *Enterobacteriales*. Although it is well-known that the plant microbiome is shaped by both the soil community and the plant cultivar (Berg and Smalla, 2009), this specific colonization structure was observed for all investigated regions (Nicaragua, Costa Rica and Uganda) and cultivars ('Gros Michel', EAHBs and 'Sukari Ndizi'). This similarity indicates a rigorous plant-driven selection process of microbial colonizers. Nevertheless, specificities at lower taxonomic levels for both country and banana cultivar could be identified for each microenvironment. The most considerable differences between the sample sets were observed for the rhizosphere communities, representing the most probable source of all other plant colonizers. Based on the differing soil microbiomes combined with a plant cultivar-specific selection, disparities were found for all investigated microenvironments, whereby above-ground plant parts shared higher similarities (Figure 1).

AGROFORESTRY LEADS TO INCREASED PLANT HEALTH VIA SHIFTS WITHIN THE MICROBIOME

Within recent years, it has been shown that agroforestry systems are able to enhance soil fertility and productivity by improving certain soil physical properties and protective functions, such as nutrient cycling and carbon sequestration (Montagnini and Nair, 2004; Udawatta et al., 2009). With respect to the cultivation of bananas, it was for instance noted that Fusarium wilt epidemics in agroforestry systems are generally lower than in intensive monocultures. Undeniably, it can be assumed that these environmental benefits are associated with soil microbial activity and soil biological parameters.

The impact of agroforestry on the banana-associated microbiome was investigated on smallholder farms in Nicaragua and Costa Rica cultivating 'Gros Michel' in *Coffea* intercropping systems with and without agri-silvicultural production of different Fabaceae trees (*Inga* spp. in Nicaragua and *Erythrina poeppigiana* in Costa Rica) (Köberl et al., 2015). Before sampling, each site had remained under the respective production system for more than 50 years. Considering the entire gammaproteobacterial microbiota, the banana-colonizing community was found to be highly stable, especially in the endophytic niche, and no significant differences in the overall structure and diversity could be observed. However, indicator species for each production system could be identified. In general, banana plants grown in agroforestry systems were characterized by an increase of potentially plant-beneficial bacteria, like *Pseudomonas* and *Stenotrophomonas*, and on the other side by a decrease of *Erwinia* (Köberl et al., 2015). Hence, as a result of legume-based agroforestry, the indigenous banana-associated gammaproteobacterial community noticeably shifted. Green manures have so far been considered beneficial in agriculture only due to fixed nitrogen provided by the legume-associated symbiosis. However, this study demonstrated the significance of neighboring plants in shaping the plant microbiome and thereby possibly affecting plant health and productivity. This means that a sophisticated design of manipulating plant microbiomes can help reduce pesticide and fertilizer use (Pirttilä, 2016).

GAMMAPROTEOBACTERIAL HEALTH INDICATORS PREVENTING FUSARIUM WILT INFESTATION

Pests and diseases are frequently referred as one of the most limiting factors for banana production worldwide (Jones, 2000; Dita et al., 2013). Fusarium wilt (FW), caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) and also known as Panama disease, is particularly devastating, because Foc produces chlamydospores – survival structures that remain in the soil for decades without suitable hosts (Ploetz, 2006, 2015). In the absence of marketable resistant cultivars, effective management practices to reduce the impact of Fusarium wilt on susceptible cultivars are urgent (Butler, 2013; Kema and Weise, 2013). As Foc is a soil-borne pathogen, relevant steps for disease conduciveness or suppressiveness are soil- and rhizosphere-driven, but also natural banana endophytes are a promising barrier in the pathogen defense (Berg et al., 2016). Therefore, understanding plant-microbiome interactions is critical to developing successful management practices.

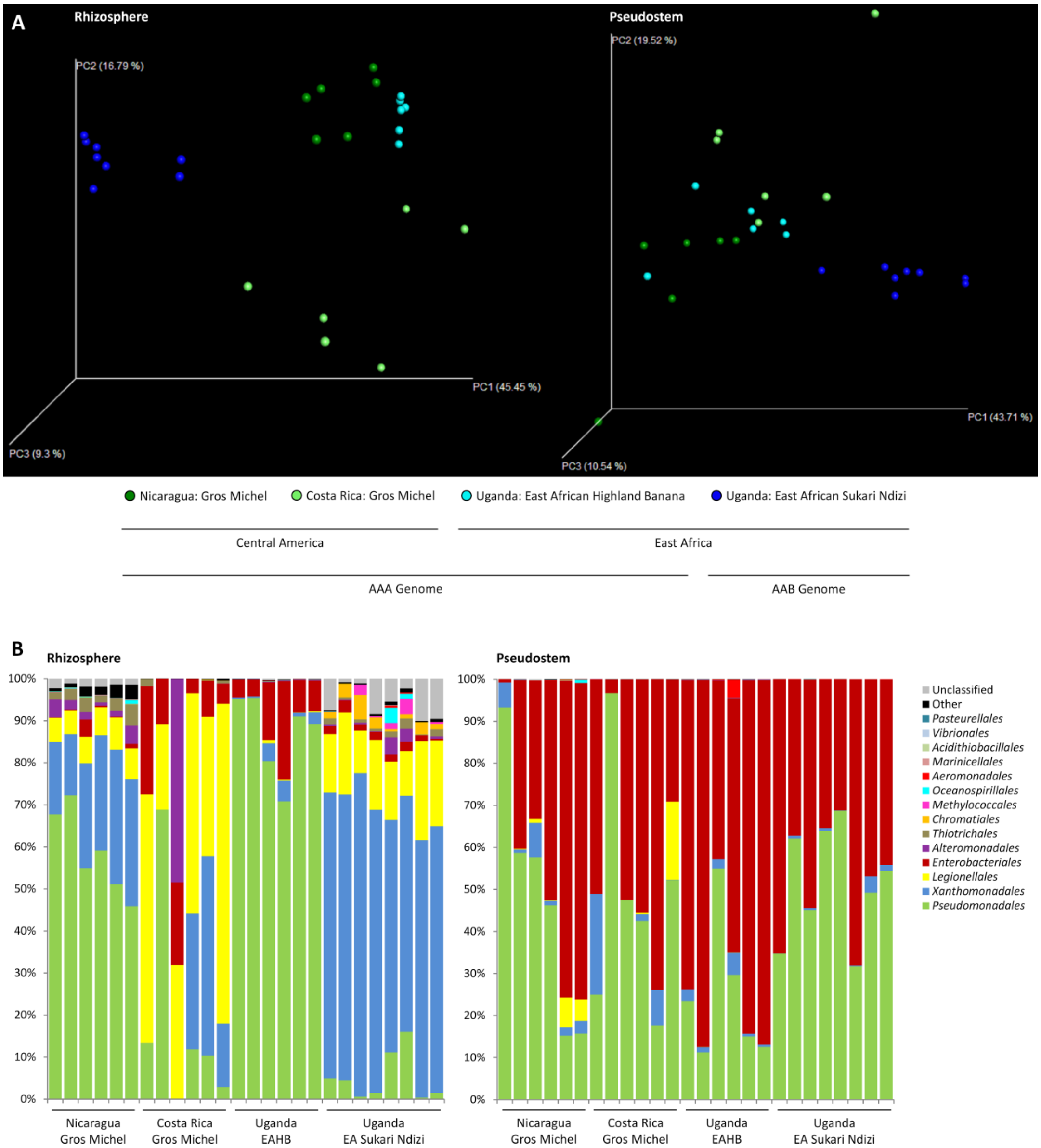

Figure 1. Principal coordinate analysis (PCoA) plots based on weighted UniFrac distances (A) and taxonomic composition at order level (B) of the gammaproteobacterial communities inhabiting rhizosphere soil and pseudostem of banana plants cultivated in different growing regions. Data were ascertained by Illumina MiSeq sequencing of gammaproteobacterial 16S rRNA gene amplicon libraries. (A) PCoA plots indicate grouping of samples by sampling region (Central America and East Africa) and banana genotype (AAA and AAB genome).

Comparative microbiome analyses performed between healthy and diseased 'Gros Michel' plants on Foc race 1-infested farms in Nicaragua and Costa Rica revealed significant shifts in the gammaproteobacterial microbiome (Köberl et al., 2017). Although substantial differences in the banana microbiome were found between both countries and a higher impact of Foc on farms in Costa Rica than in Nicaragua, the composition especially in the endophytic microhabitats was similar and the general microbiome response to FW followed similar rules. Gammaproteobacterial diversity and community members were identified as potential health indicators, which are involved in the process that keeps banana plants FW-free in Foc-infected areas. Healthy plants revealed an increase in potentially plant-beneficial *Pseudomonas* and *Stenotrophomonas*, which were also found in increased abundances in the microbiome of banana plants grown under legume-based agroforestry conditions, where a lower FW incidence was noted in comparison to banana monocultures (Köberl et al., 2015; Pirttilä, 2016). In contrast, diseased plants showed a preferential occurrence of Enterobacteriaceae known for their plant-degrading capacity. Significantly higher microbial diversity found in healthy plants could be an indicative of pathogen suppression events preventing or minimizing disease expression (Lian et al., 2008). This first study examining banana microbiome shifts caused by FW under natural field conditions opens new perspectives for the biological control of banana FW (Köberl et al., 2017). Future biocontrol studies should consider applications of antagonistic, plant growth-promoting *Pseudomonas* and *Stenotrophomonas* spp. In addition, an increased microbial rhizosphere diversity, which is substantially correlated with a low incidence of pathogen outbreaks (Jousset et al., 2011; van Elsas et al., 2012; Berg et al., 2016), could possibly counteract FW in banana. Accordingly, all management practices potentially positively impacting microbial soil diversity, such as intercropping, agroforestry or organic soil amendments, can therefore be recommended for an improved plant health and banana crop performance.

ORGANIC MANAGEMENT PRACTICES LEAD TO INCREASED MICROBIAL SOIL DIVERSITY

Organic matter input affects both physicochemical properties of the soil and biotic factors related to the soil microbiota, such as microbial biomass, microbial diversity and community structure (Saison et al., 2006; Bonilla et al., 2015). The microbial colonization of the EAHB cultivar 'Mpologoma' (AAA genome) was investigated on three smallholder farms in Uganda, in order to reveal differences according to applied organic soil amendments. Aiming to uncover consistent shifts attributable to specific soil amendments, different combinations of mulch and manure were applied on each farm (mulch, manure, manure + mulch, non-treated control). Considering exclusively the gammaproteobacterial microbiome, significant differences in the plant-associated community composition and diversity were found between individual sampling farms, independently from added soil inputs. In general, the gammaproteobacterial microbiome was pretty stable in response to the organic matter inputs. However, for two of the investigated farms, a marginal increase in gammaproteobacterial diversity was observed for banana rhizospheres in areas treated with either of the organic soil amendments. For the same two farms, significant shifts were also detected in the structure of the rhizosphere communities, whereby single treatments with mulch or manure seem to have a greater impact than the combination. Further studies based on more holistic population microbiology are necessary to potentially extend this behavior to the entire microbial community.

XANTHOMONAS WILT-RESISTANT TRANSGENIC BANANAS REVEALED A STABLE MICROBIOME

Xanthomonas wilt (BXW) triggered by the plant pathogen known as *Xanthomonas campestris* pv. *musacearum* (Xcm) is a highly devastating disease in banana production, ranked first in the Great Lakes region of East and Central Africa (Aritua et al., 2008; Tripathi et al., 2009; Wasukira et al., 2012). Currently, there are no commercial pesticides, biological control agents or resistant banana cultivars available to bring the wilting disease under control, although rigorous cultural practices have been shown to minimize disease damage (Biruma et al., 2007; Tripathi et al., 2014; Blomme et al., 2017). Researchers have developed transgenic banana lines of 'Sukari Ndizi', which have already proven their enhanced resistance against Xcm under greenhouse (Tripathi et al., 2010; Namukwaya et al., 2012) and field conditions (Tripathi et al., 2014). These transgenic lines constitutively expressing the sweet pepper resistance genes *hrap* (hypersensitive response assisting protein) and *pflp* (plant ferredoxin-like protein) can provide a timely solution to the BXW pandemic.

To study the impact of the transgenes expression on non-target microorganisms, transgenic and control lines were grown under field conditions and their associated microbiome was investigated by a combined methodological approach (Nimusiima et al., 2015). Statistically significant differences between rhizosphere and endosphere bacterial communities were observed with respect to both composition and diversity. However, three years after sucker planting, no significant differences between transgenic lines and their non-modified predecessors were detected for their associated bacterial communities. In the performed risk assessment study, the expression of the BXW resistance genes appears to have no consequences for non-target rhizobacteria and endophytes (Nimusiima et al., 2015). Additional studies should address the microbiome stability over a longer time frame, confirm the stability in other soil types and under different management practices, and investigate the effects in the presence of the disease.

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