Impact of plant domestication on spermosphere and rhizosphere microbiome composition

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Citation

Perez Jaramillo, J. E. (2019, March 28). *Impact of plant domestication on spermosphere and rhizosphere microbiome composition*. Retrieved from https://hdl.handle.net/1887/70478

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Title: Impact of plant domestication on spermosphere and rhizosphere microbiome composition
Issue Date: 2019-03-28

Chapter 7

General discussion

The interactions between plants and microorganisms and the consequences of these interactions for plant growth and health have promoted a field of intense research. Currently, it is known that the plant-associated microbiome can provide a plethora of beneficial services, such as disease suppression, induction of systemic resistance, enhanced nutrient acquisition and increased tolerance to biotic and abiotic stresses (Mendes *et al*., 2011; Berendsen *et al*., 2018; Carrión *et al*., 2018; Hassani *et al*., 2018). Several root physiological characteristics and spatio-temporal differences in root exudation profiles have been shown to structure, at least in part, the rhizosphere microbiome (Jones *et al*., 2009; Sasse *et al*., 2017). To date, however, little is known on if and how plant domestication and the loss of genetic diversity affected the ability of plants to successfully assemble a beneficial microbiome in the rhizosphere and endosphere. The overall aims of my thesis were to i) assess the effect of domestication of common bean (*Phaseolus vulgaris*) on rhizosphere and spermosphere microbiome diversity and structure; ii) link specific root architectural traits and exudation profiles to the abundance of specific bacterial groups, and iii) evaluate how habitat expansion impacted rhizosphere microbiome assembly of common bean. In this chapter, I discuss the major results obtained as well as the different future research avenues.

Revisiting the impact of plant domestication on microbiome composition

It is well stablished that plant domestication and subsequent improvement of crop cultivars by genetic breeding caused phenotypic, genomic and metabolic changes which enabled humans to use several plant species for consumption and large-scale food production (Flint-García, 2013; Abbo *et al*., 2014; Smýkal *et al*., 2018). Many of these changes were accompanied by other inadvertent effects, such as the reduction in the genetic diversity of domesticated crop cultivars (Doebley *et al*., 2006) and the reduced ability of the domesticated crops to cope with herbivorous insects (Chen *et al*., 2015).

Regarding the impact of plant domestication on symbiotic associations between plants and soil microbes, it has been shown that mycorrhizal and rhizobial interactions were, in some cases, undermined (Hetrick *et al*., 1992; Kiers *et al*., 2007; Lehmann *et al*., 2012; Sangabriel-Conde *et al*., 2015; Ellouze *et al*., 2018; **Chapter 2**). To date, however, the effect of plant domestication on the vast majority of microbes that live in the spermosphere, rhizosphere, phyllosphere or endosphere is largely unknown. The first attempts to unveil the impact of domestication on root and rhizosphere microbial communities were performed through culture-dependent methods. For instance, Germida and Siciliano (2001) showed that the rhizosphere bacterial community of ancient landraces of wheat was more diverse than that of two modern wheat cultivars. Similarly, characterization of the endophytic bacteria in stems and roots of wild and cultivated rice showed that the number of diazotrophic endophytes was higher in wild rice than in cultivated rice (Engelhard *et al*, 2000; Koomnok *et al*, 2007). Subsequent profiling of rhizospheric bacterial and fungal communities of the wild relative of maize, *Zea mays* subsp. *parviglumis* (Balsas teosinte) and two domesticated maize cultivars showed that several bacterial diversity indices were higher for teosinte than for the domesticated cultivars (Szoboszlay *et al*., 2015). Recently, with the advent of high throughput (HTP) sequencing technologies, the taxonomic diversity of plant-associated microbiomes of several wild and modern cultivars have been determined. Most of these studies showed that the α-diversity of the plant-associated microbiomes was similar for domesticated and wild plant species (Bulgarelli *et al*., 2015; Cardinale *et al*., 2015; Shenton *et al*., 2016; Chaluvadi and Bennetzen, 2018). Nevertheless, in a study with *Agave* species, cultivated species showed a lower prokaryotic diversity than the two wild species (Coleman-Derr *et al*., 2016). Also in a study with rice, wild species showed the highest diversity of root-associated bacteria as compared to modern rice (Tian *et al*., 2017). Remarkably, it has been repeatedly found that the structure of the microbial communities associated with wild and cultivated plant species diverges as well as the abundance of certain microbial groups, which responded differently depending on the domestication status of the plant: in many cases, the abundance of certain taxa was reduced or augmented in domesticated/wild plants. Studies conducted with sugar beet (Zachow *et al*., 2014), barley (Bulgarelli *et al*., 2015) and lettuce (Cardinale *et al*., 2015) showed an enrichment of members of the Bacteroidetes in the rhizosphere of the wild relatives as compared to their domesticated counterparts (**Chapter 5**). Similarly, the bacterial genera *Flavobacterium* and *Pedobacter*, both from the Bacteroidetes phylum, were enriched in wild rice as compared to cultivated rice (Tian *et al*., 2017). For the rhizosphere of wild rice, the study by Shenton *et al*. (2016) further revealed an enrichment of bacterial taxa of the Anaerolineae and Nitrospirae families and a depletion of Saprospirae taxa as compared to rhizosphere of domesticated rice (Shenton *et al*., 2016). A differential abundance of root and rhizosphere bacterial taxa was also observed for foxtail millet (*Setaria italica*) and its wild ancestor green foxtail (*S. viridis*): while Gammaproteobacteria, Deltaproteobacteria and Firmicutes were enriched in the rhizosphere of the wild ancestor, Alphaproteobacteria was enriched in the rhizosphere of foxtail millet (Chaluvadi and Bennetzen, 2018). In contrast, Leff *et al*. (2016) did not observe a significant effect of the sunflower genotype on the assembly of rhizosphere bacterial communities but did find effects on fungal rhizosphere community composition. Accordingly, it was observed that unclassified *Chaetomiaceae*, *Olpidium* and *Mortierella spp.* had higher relative abundances in wild sunflower while modern sunflower accessions had higher relative abundaces of *Pleosporales*, *Preussia* spp., unclassified *Thelebolaceae*, *Fusarium* spp., and *Conocybe* spp. (Leff *et al*., 2016). In summary, there is an increasing number of studies that have shown a significant impact of plant domestication on the rhizosphere microbiome composition. To what extent plant phenotypic, genotypic and physiological alterations caused by domestication have contributed to these microbiome changes is yet unknown.

Phaseolus vulgaris **domestication and its impact on microbiome assembly**

In this thesis common bean was used as a plant 'model' to assess the impact of domestication on the diversity of root- and seed-associated bacterial communities. Common bean (*Phaseolus vulgaris* L.) is a very important legume crop, grown worldwide in a broad range of environmental conditions (Broughton *et al*., 2003). Common bean is a good source of protein, carbohydrate and micronutrients and represents the basis of subsistence economy for low income farmers in developing countries (Akibode and Maredia, 2011; CGIAR, 2013). Initially, using an agricultural soil collected in the Colombian highlands, I assessed the rhizosphere bacterial assembly and characterized root phenotypic traits of wild and modern common bean accessions. In summary, we observed a higher relative abundance of Bacteroidetes, mainly *Chitinophagaceae* and *Cytophagaceae*, in the rhizosphere of wild bean accessions and an increase in relative abundance of Actinobacteria and Proteobacteria in the rhizosphere of modern bean accessions. It was also possible to associate the divergences in rhizobacterial community composition between wild and modern bean accessions with differences in root morphological traits. Wild common bean accessions showed significantly higher specific root length (SRL) values as compared to modern bean accessions. Subsequently, it was shown that the SRL explained a significant portion of the variability in rhizosphere microbiome observed, specifically the abundance of families from the phylum Bacteroidetes (**Chapter 3**). Therefore we concluded that the higher relative abundance of the families *Chitinophagaceae* and *Cytophagaceae* in wild common bean accessions is explained, at least in part, by the higher SRL displayed by these accessions. It has been postulated that a high SRL may provide a higher efficiency of water search and uptake, traits that in wild beans may be pivotal to prosper and survive in dry native habitats (Toro, 1990; Comas *et al.*, 2013). The number of nodules also explained a small portion of the total variability, and it was specifically related with subtle dissimilarities between the two wild bean accessions A1 and A2. For the legume plant *Lotus japonicus,* key symbiotic genes were shown to play a major role in the assembly of bacterial communities in root and rhizosphere compartments (Zgadzaj *et al.*, 2016). Whether the symbiotic plant-microbe associations are also structuring forces of the rhizobacterial community structure in common bean remains to be investigated.

Using the same computational pipeline for processing 16S raw data from several studies, higher relative abundances of members of the phylum Bacteroidetes were also found for wild relatives of several other crop species, whereas the rhizosphere of domesticated plant accessions was more enriched in members of the Actinobacteria and Proteobacteria phyla (**Chapter 5**). Why Bacteroidetes are relatively more abundant in the root and rhizosphere compartments of wild relatives of various plant species is yet unknown. We hypothesized that their prevalence in the root compartments of wild plant species may be a phylogenetic signal associated with the presence of complex biopolymers in their root exudates. Differences in plant root exudates and root architecture between crop plants and their wild relatives may be the pivotal drivers of rhizospheric interactions (Saleem *et al.*, 2018; Sasse *et al.*, 2018). However, very little is known about the differences in exudation profiles between wild and modern plant genotypes. For wheat, it was shown that a modern variety exuded three to five times more “simple” sugars (mainly fructose, glucose, and maltose) than an ancient wheat cultivar under stress conditions (Shaposhnikov *et al.*, 2016). Also for wheat higher contents of fructose, galactose and *myo*-inositol were observed in wild and primitive genotypes, while higher contents of isomaltose, sucrose, hexadecanoic acid, octadecanoic acid and 1-octaconasol were detected in modern wheat varieties (Iannucci *et al*., 2017). Similarly, it is known that several wild plant species present differences in root architectural traits, such as root density and SRL, as compared to modern cultivars (Martín-Robles *et al*., 2015). However, very little is yet known about differences in seed and root exudation profiles as well as root morphological traits between wild relatives and modern cultivars of other plant species. Recent metabolomics techniques and analyses can be used to accurately determine the composition of exudates released by wild relatives and their modern counterparts, including targeted and nontargeted approaches. In this way, both known and unknown molecules can be captured to expand the catalogue of compounds released by plant seeds and roots.

Domestication not only encompasses changes in plant genetics and physiology but also involves habitat expansion and agricultural management. To begin to understand how these domestication factors affect the rhizosphere microbiome, the bean accessions were grown in a native and an agricultural soil from the Colombian highlands (**Chapter 4**). In general, the results indicated that the transition of common bean from a native soil to an agricultural soil led to a gain of rhizobacterial diversity and led to a stronger bean genotype-dependent effect on rhizosphere microbiome assembly. A core rhizosphere microbiome was identified and mainly consisted of genera from the Proteobacteria phylum, in particular *Rhizobium*, *Bradyrhizobium, Mesorhizobium, Burkholderia, Novosphingobium* and *Sphingomonas*. A significant portion of this common bean core microbiome is composed of bacterial genera with nitrogen fixing capabilities, an important feature of microbes associated with leguminous plant species. For other non-leguminous plant species, these bacterial genera are also members of the core rhizosphere microbiome (Peiffer *et al*., 2013; Yeoh *et al*., 2016; 2017), suggesting a homogenization of rhizobacterial diversity of plants grown in different agricultural landscapes. Co-occurrence analyses further showed a reduction in complexity of the interactions going from native to agricultural soil. Based on these results I concluded that rhizobacterial community assembly for common bean grown in agricultural soil is less complex and more modular than for common bean in native soil, making it relatively more easy for a given soil bacterial species to invade and establish in the rhizosphere of bean plants grown in agricultural soils.

For the spermosphere, we observed a decrease of α-diversity for all bean accessions as compared to bulk soil, which resembles the reduction in diversity revealed for other plant compartments (Bulgarelli *et al*., 2015). Proteobacteria, Actinobacteria and Firmicutes were enriched in the bean spermosphere and small but significant differences in β-diversity were detected between the spermosphere microbiomes of wild and modern accessions. These results suggested a domestication effect on microbiome assembly already at this early developmental stage of common bean. Exudation profiles showed that the spermosphere of a modern bean accession contained higher levels of glutamate and glutamine as compared to a wild bean accession. Therefore an *in vitro* assay was performed to evaluate whether glutamate exerts a selective effect on the abundance of families from the Actinobacteria phylum. Although a strong impact on microbiome composition was observed, it was not possible to observe a clear trend towards specific bacterial taxa consistently enriched by the amino acid treatment. More specifically, the addition of glucose, glutamate or cysteine led to a reduction of the relative abundance of the Actinobacteria as compared to the minimal medium. Statistically significant effects of glutamate on the relative abundance of this phylum were not found. In order to accurately evaluate the effect of glutamate on soil bacterial communities, an optimization of glucose and amino acid levels in the culture media is needed. Furthermore, it is pivotal to validate whether glutamate is also a more abundant seed exudate in the spermosphere of modern accessions germinating in soil.

Common ground and next steps in plant microbiome research

In this thesis I aimed to decipher the impact of plant domestication on the spermosphere and rhizosphere microbiome composition of common bean. In an effort to go a step further I tried to link those particular assemblies with plant genotypic and phenotypic traits, in particular root phenotypic traits and specific exudates of wild and modern accessions of common bean (**Chapters 3 and 6**). Several exudates and hormones such as malic acid (Lakshmanan *et al*., 2012), citric acid (Zhang *et al*., 2014), salicylic acid (Lebeis *et al*., 2015; Berendsen *et al*., 2018), ferulic acid (Beckers *et al*., 2016), coumarins (Stringlis *et al*., 2018) as well as plant emitted volatile organic compounds (Rasmann *et al*., 2005; Schulz-Bohm *et al*., 2018) have been proposed as modulators of plant microbiome assembly. In a recent study, root exudates collected from *Avena barbata* were used to grow *in vitro* several typically rhizosphere-associated bacterial isolates and the authors observed a preference of these bacteria for consumption of aromatic organic acids such as nicotinic, shikimic, salicylic, cinnamic and indole-3-acetic (Zhalnina *et al*., 2018). In this thesis, it was observed that modern accessions of common bean showed higher exudation of the amino acid glutamate and I tried to link the higher abundance of this amino acid with the abundance of Actinobacterial families (**Chapter 6**). Despite these efforts, and that several exudates have been proposed as pivotal microbial regulators, validation that the exudate is produced and released into the soil by plant roots is required. Most exudate characterizations are performed in hydroponic or *in vitro* systems, without taking into account the possible impact of soil structure and texture on exudation profiles. In soil, root exudates are rapidly catabolized by microorganisms in the rhizoplane and the rhizosphere. Therefore, the first step would be to use specific devices such as microsuction cups to sample root exudates directly from plant roots growing in soil (Dessureault-Rompré *et al*., 2006; Eisenhauer *et al*., 2017). Subsequently, it is important to use both targeted and untargeted metabolite analyses (Bingol, 2018) in order to characterize the broad range of root exudates, beyond the already known sugars, organic acids and amino acids that are commonly determined.

Similarly, the influence of root architecture and particular root physiological traits on microbiome assembly is still largely unknown. In this thesis, I linked the specific root length of wild common bean accessions with the abundance of the bacterial families *Chitinophagaceae* and *Cytophagaceae* from the Bacteroidetes Phylum. However, the mechanism(s) underlying the significant association of these Bacteroidetes families with roots of wild common bean was beyond the reach of the experimental setup used in this thesis. Differences in root morphological traits were also described for maize, and the root system of teosinte, which is the maize ancestor, had proportionally more fine (diameter < 0.03 mm) roots than modern varieties and it developed the highest root to shoot dry weight ratio (Szoboszlay *et al*., 2015). Thinner roots may provide a higher efficiency of water search and uptake, characteristics that are of pivotal importance in dry periods (Comas *et al*., 2013). How these root phenotypic traits affect the recruitment and colonization of microbes is largely unknown (Saleem *et al*., 2018). Additionally, most root phenotyping methods are based on seedling plants, and the extrapolation of this data to predict the architecture of a mature root system is problematic (Zhao *et al*., 2017). Therefore, the first step to decipher the effect of root architectural traits on microbiome assembly is to perform phenotyping of root systems in rhizotrons suitable for plant growth in controlled conditions and non-invasive image acquisition of root systems (RhizoTubes) (Jeudy *et al*., 2016). Subsequently, and using the same phenotyping system, sampling of specific root regions, such as root tips, lateral roots and mature root zones, should be performed to get a better insight into the spatial distribution of root microbiomes. Characterization of microbial communities through metataxonomic and metagenomic approaches associated with these regions, in plants grown expressing their natural root plasticity, can unveil the diversity and functional repertoire of microbes as a function of root phenotypic traits.

The core microbiome and the search for heritable traits

In this thesis we also aimed to find bacterial taxa differentially abundant in wild common bean and in modern accessions. Additionally, we looked for a particular set of microbes that could be part of a microbial core. Accordingly, we found that the bacterial genera *Rhizobium*, *Bradyrhizobium, Mesorhizobium, Sphingomonas,* and *Streptomyces* were part of highly abundant taxonomic core that was shared by all eight bean accessions in the agricultural and in the native soils tested in this thesis. A significant portion of the core was composed of bacterial genera with nitrogen fixing capabilities. Several studies have also characterized the core microbiome for other non-leguminous plant species and the same rhizobacterial genera that made part of the common bean core were found as abundant core members (Peiffer *et al*., 2013; Yeoh *et al*., 2016; 2017; Chen *et al*., 2018; Walters *et al*., 2018). Recently, it has been proposed that core microorganisms may help organizing the assembly of resident plant-associated microbiomes within and around host plants (Toju *et al*., 2018). In the same perspective paper, it was proposed that one strategy for plant microbiome management is the core reinforcement strategy. Briefly, it is proposed that co-inoculations of core microbes with complementary roles may help increase plant physiological homeostasis, promoting stable establishment of late colonizing microorganisms (Toju *et al*., 2018). In this sense, future research needs to focus on the isolation and functional characterization of microbial species that are part of the core, and on the evaluation of how the re-introduction of these core microbiome members helps plants to overcome biotic and abiotic stresses. For common bean, I managed to establish an initial bacterial collection that was highly representative for the dominant taxa identified in the core spermosphere. These results suggest that a substantial proportion (66.6%) of the bacteria detected as part of the core microbiome by NGS, presenting relative abundances higher than 0.5%, are indeed culturable contradicting the old paradigm that only 1-5% of the microbes detected in soil and plant-associated environments is culturable.

Finally, the search for heritable plant traits that can be exploited in breeding strategies is one of the main priorities in present and future plant microbiome research. One of the first attempts to identify plant traits and genes involved in beneficial interactions with soil microorganisms was done using recombinant inbred line (RIL) populations, derived from a cross between a cultivated tomato and a wild tomato relative (Smith *et al.*, 1999). In that study, it was shown that three quantitative trait loci (QTL) were associated with disease suppression by the biocontrol strain *Bacillus cereus* UW85, and three other QTL were associated with the growth of *B. cereus* on the seed (Smith *et al.*, 1999). A more recent study provided, for the first time, insights into genomic regions in the model plant Arabidopsis associated with phyllosphere microbiome composition. More specifically, loci responsible for defense and cell wall integrity were linked to variations in the composition of the microbiome of Arabidopsis leaves (Horton *et al.*, 2014). Furthermore, genetic loci have been identified for their involvement in the ability of Arabidopsis accessions to respond to the activity of plant growth-promoting bacteria (Wintermans *et al.*, 2016). Mendes *et al.* (2018) showed that resistance breeding in common bean against *Fusarium oxysporum* also had unintentionally co-selected for plant traits that support rhizosphere microbes with potential antagonistic activities to this fungal root pathogen. However, the genetic basis for microbiome assembly in the different ‘spheres of crop plant species is still largely unknown. Therefore, further research should focus on the characterization of microbial communities together with genome-wide association mapping strategies. Genome-wide association studies traditionally rely on plant populations with hundreds of accessions in order to associate specific regions in the genome with phenotypic traits of interest. Accordingly, studies with a significant number of well characterized RIL populations must be designed and a detailed microbial characterization, taxonomically and functionally, of a specific plant compartment at an appropriate developmental stage must be performed. Ideally, genomic regions associated with the abundance of specific rhizobacterial families or functional microbial genes can be pinpointed in the plant genomes. In this way, microbiome-based breeding programs would include specific genomic regions responsible for the recruitment of specific bacterial groups with beneficial properties, such as diazotrophic bacteria, plant growth promotors or biocontrol agents.

Concluding remarks

The research presented in this thesis indicated that domestication of common bean had a significant effect on the abundance of specific bacterial groups in the rhizosphere and in the spermosphere. These differences may be due to divergent root phenotypic traits and/or different exudation patterns. These genotype-dependent effects were primarily visible in an agricultural soil, where the soil physicochemical conditions and the resident microbial communities were optimal for common bean growth. The higher abundance of Bacteroidetes in the rhizosphere of wild accessions as well as the higher abundance of Actinobacterial families in the rhizosphere and spermosphere of modern accessions were among the specific trends observed in this study. Intriguingly, several families within Bacteroidetes have been also found enriched in and on roots of other wild plant species. What the underlying mechanisms are that drive compositional and functional differences between the microbiomes of wild relatives and modern accessions should be the focus of future research, including studies addressing the functional impact of these microbiome shifts on plant growth, development and health.