

Chapter 44

The Edible Plant Microbiome: Importance and Health Issues

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Abstract Plants live together with microbial communities to form tight interactions that are essential for the performance and survival of the host. In recent decades, many studies have discovered a vast plant-associated microbial diversity. However, even though plants are a substantial part of a balanced diet including raw-eaten vegetables, fruits and herbs, the plant-associated microbial diversity has been largely ignored in this context. We hypothesize that the edible plant microbiome and its diversity can be important for humans as (i) an additional contributor to the diversity of our gut microbiome, and (ii) as a stimulus for the human immune system. Two specific examples for plant microbiomes, of lettuce and banana, are discussed in comparison with other relevant studies to explore these hypotheses. Moreover, the biotechnological potential of the edible plant microbiome is evaluated.

44.1 Plant-Associated Microbial Diversity

All Food Plants are Associated with a High Diversity of Microorganisms This diversity is still currently only partly characterized and is, to a certain degree, specific for the host species or even cultivars of food plants (Berg and Smalla 2009). This diversity is also specific for each microhabitat of plants which are usually distinguished as: the rhizosphere (roots), the phyllosphere (leaves), the caulosphere (stem), the anthosphere (flowers), the carposphere (fruits), and the endosphere

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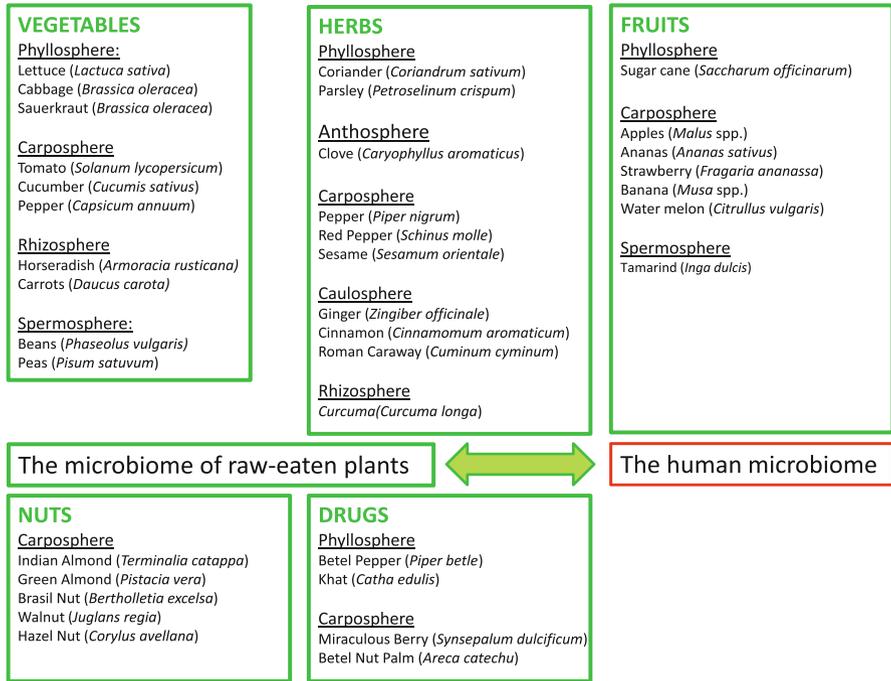


Fig. 44.1 Interactive microbiomes. Examples of the edible plant microbiome of fruits, herbs, nuts and drugs. We eat all parts of the plants including the phyllosphere (*lettuce, cabbage*), the rhizosphere (*carrots, turnip*), the carposphere (*tomato, banana*) as well as seeds (*beans, peas*) including all endospheres

(all inner parts). Although the discovery of specific microbiomes is primarily associated with the rhizosphere, there are currently only a few other compartments where species-specific diversity was detected, e.g. the carposphere (Leff and Fierer 2013). Given the estimated number of 370 000 species of higher plants, a great deal of work is still required before the details of global plant microbiome diversity will be fully understood.

Plants are a basic and substantial part of our daily diet. Vegetables, fruits, herbs, nuts, and medicinal herbs belong to the raw-eaten plants; several examples of each group are shown in Fig. 44.1. Our food thus comprises all parts of plants that include their microbial habitats and microhabitats that can be colonized by up to 10^4 – 10^{10} microorganisms per gram of plant. These microbial habitats include the phyllosphere (lettuce, cabbage), the rhizosphere (carrots, turnip), the carposphere (tomato, banana), as well as the seeds (beans, peas) and corresponding endospheres. An initial study published by Leff and Fierer (2013) demonstrated that fruits and vegetables harbored distinct and diverse bacterial communities, and interestingly showed that vegetables or fruits grown primarily close to the soil surface (i.e., sprouts, spinach,

lettuce, tomatoes, peppers, and strawberries) appear to share communities characterized by high relative abundance of *Enterobacteriaceae*. The authors concluded that humans are exposed to substantially different bacteria depending on the types of fresh produce they consume.

What could the effect of this exposure be on humans? Plant-associated microorganisms could have both a direct and indirect influence on human health. Indirect positive effects are linked to organisms that enhance the quality factors (including the content of active principles). Only a few examples are known for such effects, and most are related to medicinal plants and their bioactive substances (Köberl et al. 2013). For example, microorganisms are involved in the production of antimicrobial substances, e.g. taxol in endophytic fungi of *Taxus baccata* (Garyali et al. 2013), apigenin in *Chamomille matricaria* (Schmidt et al. 2014), or maytansine in *Putterlickia verrucosa* (Wings et al. 2013). Moreover, fruit-associated bacteria seem to influence the aroma expression in strawberries, where *Methylobacterium* treatment has been shown to enhance the production of aromatic furaneol substances (Verginer et al. 2010a). Evidence was also provided by Verginer et al. (2010b) for an influence of grape-associated microorganisms on the aroma of wine, indicating that the “terroir” effect can to some extent be attributed to bacteria. The indirect negative effects caused by plant-associated microorganisms are well-studied. The outbreak of plant pathogens is often associated with a microbiome shift and accompanied with minor pathogens. They do not only contribute to bad odor and taste, but also to the expression of mycotoxins which are among the world’s most toxic and carcinogenic compounds (Wu et al. 2014). They have been responsible for numerous foodborne diseases and epidemics throughout history including *Claviceps purpurea*, the causative agent for the infamous Saint Anthony’s Fire in Medieval times that occurred after eating contaminated bread (Belser-Ehrlich et al. 2013). Although such problems could be primarily solved by food hygiene, *Fusarium* mycotoxins still play an important role for our health (Wu et al. 2014). There is still very little knowledge concerning the long term effects of bioactive compounds at low concentration, and only recently has evidence been introduced for endophytes that produce novel and still poorly understood compounds. New technologies will contribute to increase the detection rate of specific beneficial plant-microbe interactions that are also relevant for human health.

What do we know about the direct effects of plant microbiomes that we consume along with our food? Most of our existing knowledge concerns fermented food, such as yoghurt as the foremost example for sources of probiotic strains. However, a substantial part of our plant diet is consumed fresh and may possibly include trillions of microorganisms during each meal. Even after washing or rinsing food surfaces, a substantial number of bacteria is expected to enter the body with our food. Our primary hypothesis is that the edible plant microbiome and its microbial diversity is important for humans as: (i) a contributor to the diversity within our gut microbiome, and (ii) as a stimulus for our immune response. We will present two examples for crop-associated microbiomes which are eaten raw by humans: of lettuce and banana. Furthermore, we will discuss our hypotheses as well as the impact of microbial diversity in general.

The Specific Structure of the Lettuce Microbiome Lettuce species such as *Lactuca sativa* L., *Eruca sativa* Mill., and their varieties belong to the most important raw-eaten vegetables world-wide and are a substantial part of a balanced, healthy diet. Several beneficial effects on health and lifestyle are attributed to the consumption of lettuce as it contains several vitamins, and is also a source of manganese and high amounts of dietary fibers. The relatively low amount of carbohydrates and fats correlates with its low calorie value. Lettuce provides habitats for a diverse range of microbes (Rastogi et al. 2012; Rastogi et al. 2013). Lettuce-associated microorganisms have currently only made it into the headlines in the context of scattered pathogen outbreaks. There are two crucial features that may be responsible for lettuce's vulnerability to pathogens: the variability and specificity of the associations within the microbial communities. Overall, a proportion of 12.5 % cultivar-specific bacteria were identified for the rhizosphere of eight different *Lactuca sativa* cultivars as well as the wild relative *L. serriola*. In addition, a large core microbiome was identified that includes 68 operational taxonomic units from nine major phyla (*Proteobacteria* the most abundant), and represents 48.8 % of the microbiome. A correlation analysis showed that within the lettuce microbiome co-occurrence prevailed over co-exclusion. Although predominant taxa (e.g. *Pseudomonas*, *Flavobacterium*, and *Sphingomonadaceae*) showed positive interactions, they were not necessarily involved in highly correlated modules of species. This loose bacterial network observed for lettuce allowed allochthonous organisms to colonize lettuce to interactive niching in microbial communities.

Little is known about the impact of biotic factors on the lettuce microbiota. Our hypothesis was that any disturbance of the native microbiomes (i) can induce drastic shifts in the community and that each pathogen outbreak (ii) could be accompanied by “minor”, less virulent pathogens. In mesocosm and field experiments by using a combined approach including network analyses of 16S rRNA gene amplicon libraries and FISH microscopy (see Chap. 31), we found substantial impacts detectable as microbiome shifts by a plant pathogenic fungus, herbivorous gastropoda, or visiting pets. Although the genera *Enterobacter*, *Stenotrophomonas*, *Pseudomonas*, and *Acinetobacter* form a core microbiome, all three disturbing factors induced significant shifts in the community and increased species richness. In *Lactuca*, this was strongly correlated with an increase of *Enterobacter* and in *Eruca* with *Escherichia/Shigella* and *Pantoea*—all genera contain potential pathogens. A bacterial diversity associated with leaves is detectable by cultivation and bacterial DNA analysis, but very few bacteria are detected on the surface as only a few colonies occupy cavities along the external surface and in the vicinity of stomata (Fig. 44.2a). Through colonization experiments, we revealed unexpected colonization patterns of enteric species in lettuce leaves and found that bacterial populations do not colonize the surface, but rather intrude into the endosphere (Fig. 44.2b).

The Specific Structure of the Banana Microbiome Bananas and plantains are among the most important crops in the tropics and sub-tropical regions world-wide. Microhabitat-specific microbial communities for the rhizosphere, phyllosphere, and endosphere of bananas grown in three different traditional farms in Uganda were

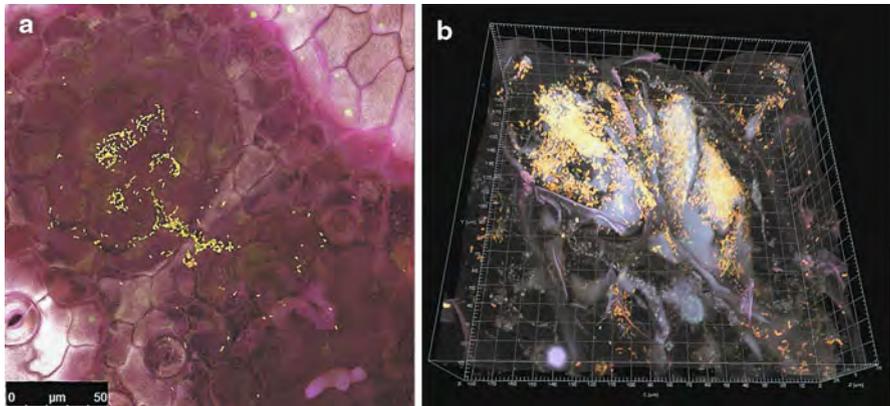


Fig. 44.2 Visualization of the lettuce microbiome. **a** Naturally occurring Gammaproteobacterial micro-colonies on the lettuce surface and in the vicinity of stomata visualized by FISH and CLSM. **b** Colonization patterns on lettuce leaves treated with *E. coli* cells. Both experiments are explained in detail in Erlacher et al. (2014)

detected (Rossmann et al. 2010). Interestingly, the banana stem endosphere showed the highest bacterial counts (up to 10^9 gene copy numbers g^{-1}), and *Enterobacteriaceae* provided 1/3 of the total bacteria. They comprise 14 genera including potential human pathogens, (*Escherichia*, *Klebsiella*, *Salmonella*, *Yersinia*) plant pathogens (*Pectobacterium*), but also disease-suppressive bacteria (*Serratia*). This dominant role of enterics can be explained by their permanent nature and the vegetative propagation of banana plants, as well as the addition of human and animal manure in traditional cultivations.

44.2 The Edible Plant Microbiome: Diversity and Human Health

Concerning our first hypothesis of a link between the plant and human gut microbiome, there is an interesting overlap between the plant and human gut microbiome with respect to species composition and function (Ramírez-Puebla et al. 2013). Recent studies showed that the stomach is colonized by a higher diversity of microbial species than has long been expected, and explained by the hostile conditions of low pH values. The stomach milieu thus does not pose a strict barrier for microbial passage as was previously thought (von Rosvinge et al. 2013). Even though the effects of probiotics are often controversially discussed, it has now been shown that strains, including probiotics, survived the stomach passage to establish successfully in the gut (Iqbal et al. 2014). David et al. (2014) also recently provided additional evidence for the survival of foodborne microbes (both animal- and plant-based diet)

after transit through the digestive system, and that foodborne strains may have been metabolically active in the gut.

Our second hypothesis is that bacteria, associated with our diet, such as *Enterobacteriaceae*, act as stimuli for our immune system. Recently, Hanski et al. (2012) showed a correlation between bacterial diversity and atopy as shown through significant interactions with *Enterobacteriaceae*. Furthermore, they showed a positive association between the abundance of *Acinetobacter* and Interleukin-10 expression in peripheral blood mononuclear cells in healthy human individuals. Interleukin-10 is an anti-inflammatory cytokine and plays a central role in maintaining immunologic tolerance to harmless substances (Lloyd and Hawrylowicz 2009). Endotoxin derived from Gram-negative bacteria, such as *Enterobacteriaceae*, is known to have allergy-protective and immunomodulatory potential (Doreswamy and Peden 2011).

Microhabitats of plants are a reservoir for *Enterobacteriaceae* (Leff and Fierer 2013, Rastogi et al. 2012), which also include potentially human pathogenic bacteria such as human enteric pathogens (Brandl 2006). Particularly after intermediate disturbances, these human enteric pathogens are enhanced (Erlacher et al. 2014). Although outbreaks of enteric pathogens associated with fresh produce in the form of raw or minimally processed vegetables and fruits have recently increased (Holden 2010), the ecology of enteric pathogens outside of their human and animal hosts is less understood (Teplitski et al. 2011). If plants are a natural reservoir of *Enterobacteriaceae*, then these bacteria must have been a “natural” part of our diet for a long time. Taking into account how many vegetables and fruits are eaten by people worldwide, these outbreaks seem to be more of an accident than the norm, particularly considering that traditionally, food was not processed and sterilized before eating. A function of the plant-associated microbiome as an immunostimulant or “natural vaccination” is more likely than their pathogenic role.

44.3 Conclusions

Members of the prokaryotic and eukaryotic domains of life are often tied together by intricate interactions. While past research has paid much more attention to the pathogenic interactions, the results obtained over the last decade have taught us much more about a beneficial balance between microorganisms and their hosts (Blaser et al. 2013). It seems that in developing these interactions, diversity plays an incredibly important role. Diversity is intrinsically correlated with a low incidence of pathogen outbreaks in both plants and humans. Where does microbial diversity come from? The plants themselves as well as their secondary metabolites and microbiomes co-evolved together; microbes contribute to the diversification of plants and *vice versa* and continue to add to the high plant-associated microbial diversity. Interesting examples are medical as well as endemic plants which harbor a unique microbiome (Zachow et al. 2009; Köberl et al. 2013). Conversely, crops cultivated in intensive agriculture are often characterized by a reduced diversity in comparison with organic agriculture or natural ecosystems. In the past, breeding strategies induced a specific microbiome

as cultivar-specificity was very often reported (Berg and Smalla 2009). Our lettuce example revealed a higher diversity in comparison to its wild ancestor as well as a loose bacterial co-occurrence network in the modern cultivars. This could explain its susceptibility for pathogens as well as for biocontrol agents. Efficient biocontrol approaches were already shown for lettuce (Scherwinski et al. 2008; Erlacher et al. 2014). The enhancement of plant-associated microbial diversity is important for the sustainability of future agriculture. In addition, for human food and health, microbial diversity is an important issue, and we should take care of plant-associated diversity and produce our food in a way that is optimal for this purpose. Biotechnological strategies can be developed to contribute to this purpose. For example, “microbiome therapies” are a promising method to maintain or enhance plant-associated microbial diversity in combination with quality control (Gopal et al. 2013). Another interesting example is the biocontrol agent *Bacillus amyloliquefaciens* FZB42, which was able to enhance the overall plant-associated diversity (Erlacher et al. 2014). Next generation microbial inoculants should take both the diversity as well as human health issues into consideration (Berg et al. 2013), and someday in the future should have the potential to control plant diseases, generally enhance microbial diversity, and stimulate our immune system.

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