Review

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Antibiotic Resistomes in Plant Microbiomes

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Microorganisms associated with plants may alter the traits of the human microbiome important for human health, but this alteration has largely been overlooked. The plant microbiome is an interface between plants and the environment, and provides many ecosystem functions such as improving nutrient uptake and protecting against biotic and abiotic stress. The plant microbiome also represents a major pathway by which humans are exposed to microbes and genes consumed with food, such as pathogenic bacteria, antibiotic-resistant bacteria, and antibiotic-resistance genes. In this review we highlight the main findings on the composition and function of the plant microbiome, and underline the potential of plant microbiomes in the dissemination of antibiotic resistance via food consumption or direct contact.

The Plant Microbiomic Resistome

The plant microbiome represents a major pathway through which humans are exposed to antibioticresistant bacteria and resistance genes that are naturally present in the environment (Figure 1). Our knowledge of **antibiotic resistomes** (see Glossary) in nonclinical environments is currently increasing rapidly from the study of wastewater-treatment plants and intensive animal-feed operations, two important man-made reservoirs of antibiotic resistomes [1–4]. Studies of the potential spread of antibiotic resistance in the environment, however, have mostly focused on the evolution of antibiotic resistomes in soil and wastewater, with little attention being paid to the subsequent spread of antibiotic resistance via plant microbiomes. We highlight here the potential impact of the **plant microbiomic resistome** on human health and suggest areas that need to be investigated further to understand and minimize the risk of spreading antibiotic resistance via food consumption.

Plant Microbiomes: Who Is There?

Plants, like all eukaryotes, have developed in a microbial world and therefore do not grow as axenic organisms in nature but are populated by diverse microorganisms, known as the plant microbiome [5-7]. Microorganisms can generally colonize all plant tissues [8] in the phyllosphere [9], the aerial components of plants, as well as in the rhizosphere, the belowground habitat of plant roots [10]. Our knowledge of the plant microbiome has historically lagged behind our knowledge of the human microbiome. Advances in next-generation sequencing technologies and bioinformatic tools, however, have improved accuracy and affordability sufficiently to study the composition and function of plant microbiota [11,12]. A large and rapidly expanding body of phylogenetic information about plant microbiomes is becoming available, demonstrating that plants team up with a core range of microbial partners [13–16]. Recent studies have reported that only a few phyla (Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria) are dominant in plant microbiomes across a variety of host species such as Arabidopsis thaliana [17,18], Brassica chinensis [19], Glycine max [13,20], Vitis vinifera [21], and several tree species [22]. The presence of some microorganisms may be due to stochastic events [12], but a core group of host-adapted strains has consistently been identified in the phyllosphere [5]. Various studies have also attempted to identify core microbiota across phylogenetically distinct plants or multiple accessions of a single plant species, or that

Highlights

Antibiotic resistance is increasing in the biosphere, but the antibiotic resistome in the plant microbiome is overlooked compared to its presence in soil and water environments.

The plant microbiome can act as the interface between human and natural microbiomes, representing a crucial pathway for human exposure to environmental antibiotic resistance.

Microorganisms associated with plants may exacerbate the dissemination of antibiotic resistance via the food chain, direct contact, and globalization.

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occupy more than one plant compartment [5,13,21]. The evidence suggests a widespread 'global' core community across host species, space, and time (Table 1).

In addition to cultivation-independent methods based on profiling marker genes or metagenomic sequencing, isolates are also important for the functional validation of *in silico* predictions. The analysis of isolate genomes can overcome the limited sequencing depth, provide a genomic and evolutionary context for individual genes, and allow access to the genomes of rare organisms [23]. Whole-genome analysis of Rhizobiales isolates combined with 16S rRNA gene surveys of the root microbiota identified an evolutionary relationship between symbiotic rhizobia and root microbiota in *A. thaliana* [24], and indicated that the symbiotic rhizobia of legumes (*Lotus japonicus*) could drive the establishment of distinctive root rhizospheric communities [25].

The phyllosphere is relatively nutrient-poor and is subject to extremes of temperature, radiation, and moisture [9]. By contrast, the rhizosphere is a region rich in nutrients and diverse microbial communities, influenced by the deposition of plant **mucilage** and **root exudates** that modulate for plant–microbe interactions [26,27]. Plants actively communicate with the highly complex microbial soil communities surrounding plant roots to assemble a taxonomically limited rhizobiome [28]. For example, the root microbiota of *A. thaliana*, a common model plant for the study of molecular biology and genetics, has substantial compositional consistencies across not only multiple continental soils but also multiple *Arabidopsis* lineages [15,17,29].

Plant Microbiomes: What Do They Do?

Plants can no longer be considered as stand-alone entities; the holobiont concept suggested that plant hosts and all their constituent microbes should be considered and studied as one entity [30]. Plant fitness is therefore a consequence of the plant *per se* and its associated microbes. The plant microbiome, as the second genome of the plant, can influence host phenotypes such as growth and tolerance to pathogens, pests, and environmental stresses (Figure 1) [5,29,31–33]. The plant-root system, where nutrients are taken up by the host plants exposed to surrounding microbes, has been suggested to be functionally analogous to the animal gastrointestinal system [34,35]. An increasing body of evidence has indicated the importance of the taxonomic and genetic diversity of root microbiomes and their interactions with plants in stimulating plant growth and decreasing susceptibility to diseases caused by pathogenic fungi, bacteria, viruses, and nematodes [28,36–38]. Plant domestication, one of the biggest accomplishments in human history, may have affected the assembly and functions of rhizospheric microbiomes [39]; this deserves more attention because plants in natural environments tend to be more diverse, with potentially more beneficial microbes, and the transition to agricultural systems may have hampered beneficial interactions between plants and microbes as a result of loss of soil microbial diversity.

Plant microbiomes extend the host phenotypes that affect host reproductive fitness. Plants have likely evolved mechanisms to attract and maintain microbial populations based on their key functional capabilities by innate or induced, stimulatory or inhibitory activities, and may have thus coevolved with their associated microbes [40,41]. In addition to the direct effects on deleterious rhizospheric microbes, many rhizospheric microbiotas have elicited physically or chemically beneficial changes to enhance the defensive capacity of aboveground plant components, a process referred to as induced systemic resistance (ISR) [42,43]. ISR in leaves can be activated by specific rhizospheric microbiotas colonizing roots and is mediated by jasmonic acid and ethylene [44–46]. By contrast, salicylic acid, a key immune regulator in leaves, also drives selection from the available microbial communities to form the root microbiome, indicating that plant immune signaling and root microbiomic assembly are functionally linked, and suggesting that there may be crosstalk between above- and belowground plant components [8,28].

Glossary

Antibiotic resistome: the antibiotic resistome comprises all of the antibiotic resistance genes. It includes resistance elements found in both pathogenic and non-pathogenic bacteria.

Mobile genetic elements (MGEs):

a type of genetic material that can move around within a genome, or that can be transferred from one species or replicon to another, playing an important part in horizontal gene transfer. MGEs include plasmids, integrons, and transposons.

Mucilage: mucilage is a thick, gluey substance produced by nearly all plants and some microorganisms. It is a polar glycoprotein and an exopolysaccharide.

Plant microbiome resistome: the antibiotic resistome in plantassociated microbes, including microbes both inside and outside their tissues, in the endosphere and ectosphere, respectively.

Root exudates: the biologically active chemicals that plant roots continuously produce and secrete into the rhizosphere. Exudates include the secretion of ions, free oxygen, water, enzymes, and a wide range of carbon-containing primary and secondary metabolites.





Figure 1. Schematic Overview of the Plant Microbiome. The aboveground components of plants, collectively known as the phyllosphere, represent an inherently open and variable habitat dominated by leaves. Antibiotic resistomes associated with phyllospheres can influence human microbiomes by direct consumption and contact, or through the food chain. The belowground components of plants, especially the narrow zone of soil surrounding the plant roots, are collectively known as the rhizosphere, which contains many microbes. The rhizospheric microbiome provides the plant with nutrient acquisition, tolerance to abiotic stress, and protection against viruses and other pathogens via induced systemic resistance (ISR) and activation of the plant immune system in response to foliar pathogenic attack (immune response). The rhizospheric microbiome, however, also facilitates the spread of antibiotic resistomes and puts human health at risk from contaminated food.

Plant microbiomes may also influence ecosystem function and global biogeochemical cycles [8,10,47] by their role in extending the host genome and thus contributing to the host phenotype [5]. The diversity of the phyllospheric microbiome has been positively linked to ecosystem productivity, and this link is maintained after accounting for the contribution of host diversity [47]. Phyllospheric microbiomes contribute to a considerable proportion of biogenic volatile organic compounds emitted annually by plants to the atmosphere [48,49]. Moreover, agricultural soil can stimulate microbe-mediated denitrification and methanogenesis, thus contributing to the emission of nitrous oxide and methane, respectively [8]. These gases not only represent a loss of carbon and nitrogen from the system but also exacerbate the greenhouse effect.

Plant Microbiomic Resistome - Movement and Human Health

The application of sewage sludge and animal manure to agricultural land is a common practice for recycling nutrients and increasing crop productivity, but also represents a major pathway for



Host species	Position	Dominant phyla	Methodology	Refs
Espeletia	Phyllosphere	Proteobacteria Actinobacteria	Illumina sequencing of 16S rRNA gene	[82]
	Rhizosphere	Proteobacteria Acidobacteria	amplicons	
Arabidopsis thaliana		Acidobacteria Planctomycetes Proteobacteria		[15]
	Rhizosphere	Acidobacteria Pyrosequencing of 16S rRNA gene amplico Actinobacteria Bacteroidetes Proteobacteria		[17]
Hordeum vulgare	Rhizosphere	Proteobacteria Actinobacteria Bacteroidetes	Illumina shotgun sequencing and pyrosequencing of 16S rRNA gene amplicons	[83]
Arabidopsis thaliana	Rhizosphere	Proteobacteria Bacteroidetes Actinobacteria	Pyrosequencing of 16S rRNA gene amplicons	[14,18]
	Phyllosphere	Proteobacteria Actinobacteria Bacteroidetes	Pyrosequencing of 16S rRNA gene amplicons	[18]
Glycine max	Phyllosphere	Proteobacteria Actinobacteria Bacteroidetes	16S rRNA gene clone libraries	[13]
	Rhizosphere	Proteobacteria Actinobacteria Firmicutes	454 Metagenomic shotgun sequencing	[20]
Pinus flexilis Pinus strobiformis	Phyllosphere	Proteobacteria Bacteroidetes		[22]
Catalpa speciosa Fraxinus pennsylvanica Fraxinus americana	Phyllosphere	Bacteroidetes Firmicutes Actinobacteria Proteobacteria	Pyrosequencing of 16S rRNA gene amplicons	
Zea mays	Rhizosphere	Proteobacteria Bacteroidetes Actinobacteria	Pyrosequencing of 16S rRNA gene amplicons	[84]
Vitis vinifera	Phyllosphere	Proteobacteria		
	Rhizosphere	Proteobacteria Acidobacteria Bacteroidetes	Illumina sequencing of 16S rRNA gene amplicons	[21]
Lactuca sativa	Phyllosphere	Proteobacteria Firmicutes		[85]
	Rhizosphere	Proteobacteria Bacteroidetes Chloroflexi	Pyrosequencing of 16S rRNA gene amplicons	[86]
Oryza sativa	Rhizosphere	Proteobacteria Acidobacteria Chloroflexi	Illumina sequencing of 16S rRNA gene amplicons	[16]
	Phyllosphere	Proteobacteria Actinobacteria	454 Metagenomic shotgun sequencing,	[87]
	Rhizosphere	Proteobacteria Chloroflexi	16S rRNA gene clone libraries	

Table 1. Dominant Bacterial Phyla in Rhizospheres and Phyllospheres



Table 1. (continued)

Host species	Position	Dominant phyla	Methodology	Refs
Brassica chinensis	Phyllosphere	Proteobacteria Firmicutes	Illumina sequencing of 16S rRNA gene amplicons	[19]
	Phyllosphere	Proteobacteria Chloroflexi Actinobacteria		
Saccharum officinarum	Rhizosphere	Proteobacteria Bacteroidetes Actinobacteria	Pyrosequencing of 16S rRNA gene amplicons	[88]
Solanum tuberosum	Rhizosphere	Actinobacteria	Pyrosequencing of 16S rRNA gene amplicons	[89]

the dissemination of antibiotic resistance (Figure 2). Many studies have investigated the potential spread of antibiotic resistance in the environment via agricultural organic fertilization under both laboratory and field conditions using qPCR, high-throughput qPCR (HT-qPCR), and metagenomics. Such data have clearly demonstrated that procedures of organic production facilitate the dissemination of antibiotic resistance in soil environments [50–52], suggesting that there is an urgent need to optimize waste disposal for sustainable agriculture and waste recycling [53]. Recent studies have suggested that biochar, commonly used in soil remediation, could also be used in organic fertilizer composting and soil to reduce the level and potential spread of antibiotic-resistance genes (ARGs) [54–56].

Antibiotic resistomes in soil, water, and air have been studied extensively [2,57,58]. By contrast, only limited examples are available for plant microbiomic resistomes. The results currently available, however, have provided valuable information on antibiotic resistomes in plant microbiomes. We have summarized plant microbiomic resistomes based on current data for food-borne antibioticresistant commensal bacteria and resistance genes (Table 2). An important finding was that the abundance of antibiotic resistomes is approximately eightfold higher in organically than conventionally produced lettuce [59]. These ARGs, conferring resistance to almost all major classes of antibiotics commonly administered to animals and humans. The lettuce samples used in this study were collected directly from supermarkets and may be consumed with little processing, and the lettuce thus poses a substantial risk for the transfer of antibiotic resistance to humans. ARGs assessed by PCR-based methods, however, cannot provide information about their functionality and mobility. More studies using culture-dependent and metagenomic approaches to investigate a broad range of vegetables are therefore still necessary to reach general conclusions. Interestingly, some ARGs have been detected in plant microbiomes but not in their surrounding environments, including soils, suggesting an intrinsic antibiotic resistome in the plant microbiome [19]. This result is important for establishing a baseline of the antibiotic resistome before evaluating its dissemination via the plant microbiome. Many of the ARGs in the plant microbiome clearly overlap with the surrounding resistome, indicating that these ARGs could be acquired and that soil resistomes might be a major source for plant resistomes [19]. Understanding the various potential pathways for disseminating antibiotic resistomes via plant microbiomes will be necessary to control or minimize the spread of antibiotic resistance.

Resistome Dissemination via the Food Chain

Food consumption represents a major route for exposure of the human microbiome to the natural microbiome; the plant microbiome acts as a bridge connecting these two microbiomes. Previous studies suggested that the daily consumption of food products such as raw leafy





Figure 2. Schematic Overview of the Effects of Organic Fertilization on Plant Microbiome Resistomes. Organic fertilization may directly increase the level of soil antibiotic resistance from manure-borne antibiotic-resistant bacteria and indirectly by the selection/coselection pressure on soil microorganisms by antibiotics and heavy metals in organic fertilizers. Antibiotic resistomes from non-pathogenic bacteria may be transferred to human pathogens by horizontal gene transfer via mobile genetic elements (MGEs), and this may compromise the efficacy of clinical antibiotics.



Host	Major findings	Methodology	Refs
Lactuca sativa Lycopersicon esculentum Raphanus sativus Daucus carota	 (i) All targeted genes were detectable on vegetables in the presence of sewage but were not detectable on vegetables in unamended soil (ii) No targets were more abundant on a vegetable grown in the presence versus the absence of sludge 1 year after the application of sewage sludge 	Culture-dependent method and quantitative PCR	[90–92]
Lactuca sativa	 (i) Over 100 unique ARGs were detected in the phyllosphere and foliar endophytes of market lettuce (ii) Organic lettuce harbored more ARGs than conventionally produced lettuce 	High-throughput quantitative PCR	[59]
Cucumis sativus Capsicum annuum Lactuca sativa Lycopersicon esculentum Raphanus sativus Daucus carota	 (i) Soil receiving manure was enriched in antibiotic- resistant bacteria and ARGs, but the abundance of ARGs in vegetables grown in manure-fertilized soil did not correspondingly increase (ii) Numerous ARGs were detected on vegetables grown in control soil. Only a small number of ARGs were additionally detected on vegetables grown only in manured soil 	Culture-dependent method and quantitative PCR	[93]
Brassica chinensis	 (i) Detected ARGs were enriched after application of struvite (ii) Twenty-five ARGs were innate in the phyllosphere but were not detectable in soil (iii) The bacterial taxa shared between rhizosphere and phyllosphere were significantly correlated with ARGs 	High-throughput quantitative PCR and Illumina sequencing	[19]
Lactuca sativa	Biochar amendment can be a practical strategy to protect crops from ARGs and contamination with human pathogenic bacteria	Quantitative PCR and Illumina sequencing	[54,94]
Lactuca sativa Sonchus oleraceus	 (i) Five subtypes of ARGs (<i>sull</i>, <i>tetG</i>, <i>tetC</i>, <i>tetA</i>, and <i>tetM</i>) and a class I integron-integrase gene (<i>int1</i>) were detected in both endophytes and phyllospheres (ii) Planting can affect the distribution of ARGs in manure-amended soil 	Quantitative PCR	[64]
Zea mays	 (i) Long-term application of sludge and manure increased the abundance and diversity of ARGs in the maize phyllosphere (ii) The maximum enrichment was 2638-fold for <i>amp</i>C-06 	High-throughput quantitative PCR and Illumina sequencing	[65]
Eruca sativa Coriandrum sativum	 (i) A diverse array of self-transmissible multiple resistance plasmids was detected in bacteria associated with produce (ii) Cultivation-independent methods are not always sufficiently sensitive to detect transferable resistomes in rare microbiomes 	Culture-based method Quantitative PCR	[79]

Table 2. Recent Findings for Antibiotic Resistomes in Plant Microbiomes

vegetables and fruits is likely a major route for the dissemination of antibiotic resistance to humans [60,61]. Plants can take up antibiotics from soil amended with animal manure [62], an organic fertilizer commonly used in agriculture, and this may apply selection pressure on the plant microbiome and contribute to the emergence and spread of antibiotic resistance to the human food chain. For example, HT-qPCR has identified hundreds of ARGs in the plant



microbiome, and organic fertilization has markedly increased the occurrence of ARGs, suggesting that plants can acquire exogenous ARGs [19].

Most ARG-carrying plant microbes are fortunately non-pathogenic [60], but their potential involvement in the spread of ARGs to other bacterial genera, including human pathogens, by horizontal gene transfer (HGT) via **mobile genetic elements** (MGEs) is a concern [63] (Figure 2). The class I integron-integrase gene (*intl1*) and genes encoding transposases are common in harvest vegetables, indicating the possibility of HGT in the phyllosphere [64,65]. The phyllosphere and rhizosphere may be key areas for HGT in plant and soil habitats because of the high possibility that cells cluster, forming biofilms in the phyllosphere, and bacterial metabolic rates and the mobility of bacteria and MGEs are high in rhizospheres [66].

Resistome Dissemination via Urban Green Spaces

Humans are increasingly less in touch with nature owing to rapid urbanization, and visiting urban green spaces such as parks, forests, green roofs, streams, and community gardens is widely promoted for city dwellers [67]. A growing body of evidence suggests that contact with nature in general, particularly contact with urban greenery, has beneficial health effects [68–71]. Urban green space has been hypothesized to be where the human microbiome can be rewilded [72], which would improve human health and wellbeing. This hypothesis is based on the assumption that a more diverse microbiome is beneficial to human health [73]. Waste discharge, such as irrigation with reclaimed water, can nevertheless increase antibiotic resistance in urban green spaces (Figure 3). For example, irrigation using reclaimed water has enriched ARGs in urban park soils, and >100 unique ARGs were greatly enriched in these soils, with as much as 8000-fold enrichment of a single gene [74]. The use of reclaimed water is likely



Trends in Plant Science

Figure 3. Scenario of a Plant Microbiomic Antibiotic Resistome. Direct contact and food chains represent major pathways by which human microbiomes are exposed to environmental resistomes. The dissemination of resistomes via (A) the food chain and (B) irrigation using reclaimed water should be given equal weight and may exacerbate the dissemination of resistomes worldwide via plant resistomes contained in their microbiomes.



to increase in the near future owing to the shortage of water needed for urbanization. Human exposure to antibiotic resistomes in green spaces (via the phyllosphere) should therefore be monitored, for example for the antibiotic resistomes of human skin microbiomes.

Resistome Dissemination via Food Processing, Preservation, and Transportation

Food is often processed and preserved to obtain safe products, extend shelf lives, and maximize profits. These processes include drying, heating to reduce water content, cooling, acidification, modified-atmosphere packaging by decreasing oxygen and increasing carbon dioxide, freezing, mild pasteurization, and UV irradiation [75,76]. The effects of these processes on bacterial flora depend on the combination, type, and conditions of the technologies used. Most processing methods can reduce the number of food-borne bacteria and thus the potential risks of bacterial contamination. For example, freezing and UV irradiation can stress or damage bacterial cells, and ultimately kill or inactivate the bacteria [76]. The dead bacterial cells, however, can be lysed due to cell-wall damage. DNA, including possible ARGs, is consequently released into the environment. The transformation of extracellular DNA plays an important role in HGT and the dissemination of antibiotic resistance in the environment [77,78]. A recent study identified a diverse array of self-transmissible plasmids conferring tetracycline resistance in vegetables purchased from supermarkets, including mixed salads, arugula, and cilantro. More importantly, the exogenous capturing of these plasmids by *Escherichia coli* indicates that they could also be potentially transferred to gut bacteria [79].

The movement of microorganisms has historically mainly been due to natural factors, for example physical forces including air and water currents, or even natural events [80]. Globalization has dramatically affected these dynamics (Figure 3), and cheap and highly efficacious long-range transport systems allow many transnational corporations to manufacture food products in regions with less-expensive labor, using ingredients from around the world, and then distribute them worldwide [81]. Bacteria associated with plants cannot be completely eliminated during food production, storage, processing, and packaging, and are consequently transported with their host material by global trade, which has a profound impact on the evolution and dissemination of antibiotic resistomes, and may cause unpredictable infections. For example, outbreaks of Salmonella poona infections in the USA associated with eating melons imported from Mexico have been linked to unhygienic irrigation and packaging at the source farms [81]. The situation is exacerbated by the cultivation of non-indigenous crops in some low-income regions to meet market demands, and these crops may be more susceptible to indigenous pathogens. For example, Guatemalan raspberries became contaminated with the protozoan Cyclospora, causing outbreaks of gastroenteritis in the USA and Canada [81]. The development and implementation of national and international guidelines for assessing the biological risk of microbiomes associated with food is a strategic priority for alleviating these threats.

Concluding Remarks and Future Perspectives

The two fundamental questions about plant microbiomes – who is there, and what do they do – are becoming increasingly well defined. The available data suggest that the plant microbiome is dominated by bacteria and bacterial communities on above- and below-ground organs with a defined taxonomic structure, and is consistently composed of a few phyla, mainly Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. The bacterial communities in plant microbiomes have profound consequences on host health and functional traits, and even on ecosystem productivity and global climate change.

We advocate that more attention should be paid to the antibiotic resistome in plant microbiomes because it poses a potential public health threat by connecting the human and natural

Outstanding Questions

Is there any phylogenetic relationship between these ARG-harboring bacteria and key members of the plant microbiome?

Is there a core antibiotic resistome in plant microbiomes across phylogenetically distinct host species? Do seasonal or spatial scales (i.e., tissues where the microbiome can be found) have an impact on the resistance diversity?

How can we disentangle the relationship between human health and the antibiotic resistome in plant microbiomes?

Can plant-associated antibiotic resistomes be magnified along the food chain with increasing tropic level?

Globalization accelerates the spread of antibiotic resistance. How can we minimize the threat associated with global movements of ARGs?



microbiomes (see Outstanding Questions). Our current understanding of antibiotic resistomes associated with plants is still in its infancy, but experimental evidence suggests that the food chain is the main route by which human microbiomes are exposed to the antibiotic resistomes in plant microbiomes and/or natural microbiomes in general. Globalization may unpredictably disseminate antibiotic resistomes associated with plants, and we believe that different types of experiments (culture-dependent and metagenomics), surveys, and international and interdisciplinary collaborations will be necessary to obtain an integrated understanding of plant antibiotic resistomes.

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